Title: Blood serum denaturation profile examined by differential scanning calorimetry reflects the effort put into ultramarathon by amateur long-distance runners

Author: Anna Michnik, Ewa Sadowska-Krępa, Agnieszka Kiełboń, Klaudia Duch, Sebastian Bańkowski

Citation style: Michnik Anna, Sadowska-Krępa Ewa, Kiełboń Agnieszka, Duch Klaudia, Bańkowski Sebastian. (2021). Blood serum denaturation profile examined by differential scanning calorimetry reflects the effort put into ultramarathon by amateur long-distance runners. "Journal of Thermal Biology" (2021), vol. 99, art. no. 103013, s. 1-7.
DOI: 10.1016/j.jtherbio.2021.103013

Journal of Thermal Biology 99 (2021) 103013

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Blood serum denaturation profile examined by differential scanning calorimetry reflects the effort put into ultramarathon by amateur long-distance runners

Anna Michnik [a], Ewa Sadowska-Krępa [b,*], Agnieszka Kiełboń [a], Klaudia Duch [a], Sebastian Bańkowski [b]

[a] Faculty of Science and Technology, Institute of Biomedical Engineering, The Silesian Centre for Education and Interdisciplinary Research, University of Silesia in Katowice, ul. 75 Pułku Piechoty 1A, 41-500, Chorzow, Poland
[b] The Jerzy Kukuczka Academy of Physical Education in Katowice, Institute of Sport Sciences, ul. Mikołowska 72a, 40-065, Katowice, Poland

ARTICLE INFO

Keywords:
Amateur runners
Differential scanning calorimetry (DSC)
Human blood serum
Protein thermal denaturation
Ultramarathon run

ABSTRACT

The impact of participation in the ultramarathon on the health and mental and physical condition is very complex. Undoubtedly, exercise brings many benefits but also involves health risks. Especially such an extreme effort as the one associated with finishing the ultramarathon run, can be dangerous to the health of the runner. With the variety of possible biomarkers of excessive fatigue that threaten health and life, a question arises which of them are the best and which should be considered in amateur long-distance runners showing particularly high individual variability. In this study differential scanning calorimetry (DSC) has been applied to show the overall effect of the 12-h run on blood sera of participants. Serum samples were obtained from the blood of ten male amateur long-distance runners, collected before and immediately after the run. Distinct changes in the shape of DSC curves have been observed for serum after finishing the run relative to pre-race serum. Statistically significant differences between stages “before” and “after” ultramarathon running have been found for parameters of the endothermic transition associated with denaturation of serum proteins. An increase in the temperature (from 70.9 ± 0.9 to 75.8 ± 2.9 °C) and excess heat capacity (from 0.859 ± 0.201 to 1.102 ± 0.226 Jg-1 °C-1) at peak maximum, the enthalpy of serum denaturation (from 18.55 ± 6.52 to 22.08 ± 5.61 Jg^{-1}) and the first moment of the thermal transition with respect to the temperature (from the value of 67.0 ± 2.1 to 72.6 ± 2.1 °C) has been observed. These results show a clear impact of running an ultramarathon on the participant’s blood serum.

1. Introduction

Ultramarathon run is a unique sport that has been growing in popularity in recent years. An ultramarathon can be defined as any running performance lasting for longer than 6 h and/or longer than the classical marathon distance of 42.195 km (Knechtle and Nikolaidis, 2015, 2018; Scheer et al., 2020). Although the long distance puts high physical demands on the runner, the popularity of ultramarathon participation increases also among amateur athletes. It seems obvious that such extreme forms of recreation as marathons or ultramarathons demand very good physical conditions great mental preparation and involve health risks. Therefore, these sports are becoming more and more interesting for scientific research. Ultramarathoners are a group of athletes that has been little studied in terms of the physiology of systems or metabolic processes. More reliable studies have been conducted among shorter distance runners, but extrapolating conclusions from marathon runners to ultra-runners appears to be an abuse. Additionally, environmental factors such as a heat or humidity can affect the thermoregulatory and metabolic responses of long distance runners. (Rodrigues Júnior et al., 2020). Ultramarathoners differ from marathoners regarding anthropometry and training (Knechtle and Nikolaidis, 2015). Ultramarathoners are older (the average age of an ultra endurance athlete is 45 years) and have a larger weekly training volume but run more slowly during training compared to marathoners (Knechtle

* Corresponding author.
E-mail addresses: anna.michnik@us.edu.pl (A. Michnik), e.sadowska-krepa@awf.katowice.pl (E. Sadowska-Krępa), agnieszka.kielbon@gmail.com (A. Kiełboń), klaudia.duch@us.edu.pl (K. Duch), s.bankowski@awf.katowice.pl (S. Bańkowski).

https://doi.org/10.1016/j.jtherbio.2021.103013
Received 21 January 2021; Received in revised form 15 April 2021; Accepted 30 May 2021
Available online 8 June 2021

and Nikolaidis, 2018). So far, most of participants in ultramarathon events were male.

Several review papers and articles provide a brief overview of the recent literature and trends in ultramarathon running and present the current state of knowledge on the topic of physiology and pathophysiology of such extreme efforts (Knechtle and Nikolaidis, 2018; Spittler and Oberle, 2019; Tiller, 2019; Martinez-Navarro et al., 2020). Physiological, pathophysiological, and metabolic responses to ultra-endurance exercise have been also investigated in nonelite and amateur ultramarathon participants (Waśkiewicz et al., 2012; Kłapcińska et al., 2013; Hoppel et al., 2019). There is a constant increase in literature regarding ultramarathon-specific illnesses and injuries, nutrition guidelines, psychology, physiologic changes, training methods, and equipment (Hoffman et al., 2014; Hoffman, 2016; Knechtle, 2017). Scientists also tried to understand the motivation of ultramarathoners during the races and preparatory periods (Waśkiewicz et al., 2019). There is still limited understanding of the psychological characteristics of ultramarathon runners.

Ultramarathoners are prone to numerous illnesses and injuries, which range from minor skin breakdown to sudden death. After extreme running effort, the development of the Acute Kidney Injury (AKI) often appears (Kao et al., 2015). According Spittler & Oberle review article (Spittler and Oberle, 2019), changes in metabolic biomarkers and hormone levels during an ultramarathon include: elevated skeletal muscle, cardiac muscle, and liver enzymes; elevated creatinine and variable fluctuations in electrolyte levels; elevated leukocytes, iron, and ferritin; decreased erythrocytes; elevated inflammatory markers (C-reactive protein, erythrocyte sedimentation rate, interleukins); decreased testosterone and increases in other hormones. In addition, ultramarathon finishers seem to have a hypovolemic physiologic response during competition with lower diastolic blood pressure and lower oxygen saturation. Sodium imbalances may be seen in ultraendurance athletes. Several reviews have evaluated the effect of hydration status on ultramarathon running (Costa et al., 2014; Hoffman et al., 2019; Burke et al., 2019). Ultramarathon runners may develop varying degrees of both hypohydration and hyperhydration (with accompanying exercise associated hyponatremia), dependent on event duration, and environmental conditions. Additionally, the demand for water and electrolytes is an individual characteristic of the athlete. Proper management of hydration is critical for both performance and overall health in ultramarathon running.

Multiple studies have examined predictor variables for successful ultramarathon running performance (Knechtle and Tanda, 2015; Coates et al., 2020). In recent years, attention has been given to the effects of aging on endurance performance in runners (Knechtle et al., 2012; Lehto, 2016; Nikolaidis and Knechtle, 2020). In general, age 35–50 years, low body mass index (BMI), and low body fat are consistent predictors of better ultramarathon performance (Spittler and Oberle, 2019). Considering changes in blood morphology and chosen biochemical parameters in ultramarathon runners Jastrzębski et al. (2015) have concluded that organ damage and negative metabolic changes during a 100-km run occurred similarly in participants less experienced as well as in well trained runners.

In this study differential scanning calorimetry (DSC) has been used to assess potential alterations in the blood serum heat capacity profile during extremely demanding 12-h of running. The thermal denaturation profiles of serum proteins have been monitored before the run and immediately after finishing the ultramarathon. It has been hypothesized that DSC heat capacity profiles of blood serum proteome would be changed due to this extreme physical fatigue. The DSC technique provides valuable information about folding and binding interactions in proteins and the thermodynamic driving forces governing protein stability. It has been recently used successfully in analyzing heat capacity changes of physiological fluids (blood plasma/serum, cerebrospinal fluid, synovial fluid) to monitor various physiological responses in health and disease (Garbett et al., 2008, 2009; Michnik et al., 2010, 2013, 2018a, 2018b; Chagovetz et al., 2011; Todinova et al., 2011, 2012, 2018; Fekecs et al., 2012; Mehdi et al., 2013; Barceló et al., 2015; Kikalishvili et al., 2015; Krumova et al., 2015; Kędra-Królik et al., 2017; Tenchov et al., 2019; Wiegand et al., 2019; Antonova et al., 2020; Dandé et al., 2020; Ferencz, 2020). Alterations in serum protein content, changes in the protein structure, changes in protein denaturation temperature due to a ligand binding or protein-protein interactions lead to modifications of the serum heat capacity profiles detected by DSC. So, the effects of extremely demanding physical activity during ultramarathon running should be reflected in serum DSC profiles.

2. Materials and methods

2.1. Participants

Ten male amateur long-distance runners (mean age 52.0 ± 6.2 years, body height 176.9 ± 4.9 cm, body mass 73.9 ± 6.0 kg, weekly covered distance 57.4 ± 22.9 km, training history 7.3 ± 2.2 years) were recruited from participants in a 12-hr race organized by the Municipal Sports and Recreation Canter and the recreation and sports club TKKF "Jastrząb" in Ruda Śląska (Poland). All runners were non-smokers and did not intend to use non-steroidal analgesics before and during the race. They completed a questionnaire on their medical and training history and signed informed consent to participate in the study after being informed about the experimental procedures. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of the Jerzy Kukuczka Academy of Physical Education in Katowice (Poland).

2.2. Study protocol

The run took place between 7 a.m. and 7 p.m., on April 28, 2018, in the town of Ruda Śląska. The contestants were required to cover a circular route of 1.6 km as many times as they could. The average distance covered was 94.73 ± 12.97 km with an average speed 7.9 ± 1.1 km h^{-1}. The ambient temperature was 24 ± 4 °C and relative humidity 46 ± 2%. The runners ingested carbohydrate-rich food (sandwiches, cookies, fruits, and carbohydrate energy bars) every 90–120 min and fluids (water and sport beverages) every 20–25 min during the run.

2.3. Blood serum samples

Blood samples were obtained 1 h before the running (pre-run) and immediately after finishing the 12-h running (post-run) into tubes for separation of serum (BD Vacutainer™ Serum Tube, UK) and then they were allowed to stand for 30 min for blood to clot before serum was separated. Serum was stored frozen for analysis at −20 °C for a period shorter than one month without unfreezing and freezing. Total protein content was assayed using the biuret method (Randox Laboratories, UK) by means of the Hitachi 917 Modular P analyser.

For DSC experiment, serum samples were diluted 20-fold with distilled degassed water. The pH value of the diluted samples was within the range 6.5–7.0.

2.4. Differential scanning calorimetry

DSC measurements were performed using the VP DSC MicroCal instrument (Northampton, MA) in the temperature range of 20–100 °C with the heating rate 1 °C min^{-1}. A constant pressure of about $1.7 \cdot 10^5$ Pa was exerted on the liquids in the cells. Duplicate scans for each sample were collected. The calorimetric data were corrected for the instrumental baseline water–water. DSC curves were normalized for the gram mass of protein and next a linear baseline was subtracted. An apparent excess heat capacity Cp^{ex} (J $°C^{-1}$ g^{-1}) versus temperature (°C) has been plotted.

The following parameters of the observed endothermic transition have been determined: the temperature and excess heat capacity at maximum (T_m, Cp_m), the enthalpy (ΔH) of serum denaturation (calculated as the area under the endothermic peak, expressed in J g^{-1}), the width of peak in its half height (*HHW*) and T_{FM} (the first moment of the thermal transition with respect to the temperature axis (the formula is given e.g. in (Michnik et al., 2010; Michnik et al., 2013)).

2.5. Statistical analysis

Statistical analysis was performed using the Statistica 13 software. For all measures, descriptive statistics were calculated. The Shapiro – Wilk test was used to check the normality of distributions of the studied variables. Student t-test was used to compare mean values of parameters of thermal denaturation transition before and after the ultramarathon running. Pearson's correlation coefficient (r) was found to describe the relationships between the age of the participants, covered distance, speed, and serum thermal transition parameters. The level of significance was set at $p < 0.05$.

3. Results

Fig. 1 shows the mean DSC curves of blood serum collected from amateur long-distance runners before and after ultramarathon. The complex endothermic transition observed on the serum DSC curve in the temperature range 40–90 °C comes from denaturation of serum proteins. Assuming there are no significant interactions between the serum proteins, this transition is the sum of thermal transitions of the individual proteins weighted according to their mass in solution (Garbett et al., 2008). For healthy people, DSC profiles made under the same physicochemical and experimental conditions are similar. The typical calorimetric profile of serum in aqueous solution usually exhibits 3–4 local maxima (Michnik et al., 2013, 2018, 2020a, 2020b; Duch et al., 2019, 2020). The first peak (or shoulder), at about 58 °C, represents mainly a non-ligated form of albumin, the most abundant serum protein. The second peak at about 62 °C (sometimes invisible) has its source in the denaturation of haptoglobin, the acute phase protein belonging to the alpha-2 globulin fraction. The third peak at about 70 °C, frequently the most intense, represents mainly the contributions from denaturation of immunoglobulins. At about 83 °C the fourth peak (or shoulder) is sometimes visible. It represents contributions from the C_H3 domain of immunoglobulin IgG1 and/or transferrin (Garbett, 2009).

The shape of serum DSC profile visible in Fig. 1 for ultramarathon runners before the run has the characteristics of a profile observed for healthy people. The comparison of DSC curves of sera before and after finishing the 12-h ultramarathon running clearly shows significant differences. The transition shifts towards higher temperatures and its shape changes due to the great effort during a long, exhausting run. The decrease in the intensity of the transition in the low-temperature range may indicate a decrease of non-ligated form of albumin. The local maximum at about 62 °C is no longer visible, which may suggest a drop in haptoglobin levels. Outlining exothermic transition above 90 °C on the pre-run profile is not visible in DSC curve after the run. Such exothermic contribution suggests the occurrence of the process of aggregation of denatured proteins in this high temperature range.

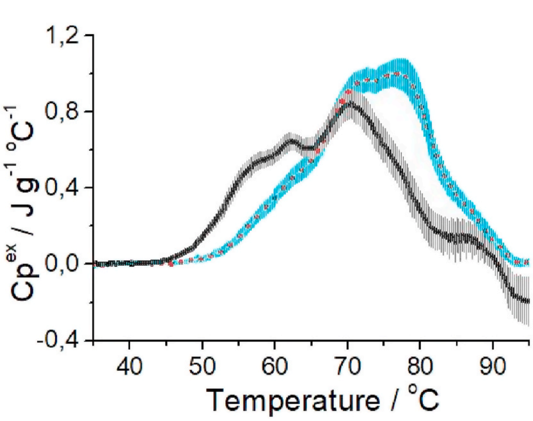

Fig. 1. Mean DSC curves of serum before (solid black line) and after ultramarathon running (dot red line); the shaded area is the standard error of the mean (SEM) at each temperature (gray for "pre-run", cyan for "post-run"). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The parameters describing the endothermic transition of serum denaturation are shown in Table 1. Statistically significant differences between "pre-run" and "post-run" stages have been found for the temperature Tm (p = 0.0003) and excess heat capacity at maximum Cpm (p = 0.00004), the enthalpy of serum denaturation ΔH (p = 0.04) and the first moment temperature TFM (p = 0.00006). The mean values of all these parameters are higher after the run. Contrary, a width of peak in its half height (HHW) decreases, but the difference between mean values of HHW in both stages is not statistically significant (p = 0.09).

Data presented in the current study refer to amateurs, which constitute a widely heterogeneous group in terms of general lifestyle and individual, not professionally designed training regimens. The Fig. 2 a-d shows the diversity of responses of ultramarathon participants to the 12-h run. Serum DSC curves before and after the run have been compared for the exemplary 4 participants.

Fig. 2a represents the set of DSC curves obtained for the 52-year-old runner who ran the longest distance (114 km) and had the highest average speed (9.5 km h^{-1}) from among all participants. Fig. 2b shows the curves for the oldest participant (63 years old) who ran 97 km with the average speed 8.1 km h^{-1}. The curves shown in Fig. 2c correspond to the 49-year-old runner who covered a similar distance to a) (111 km) with the average speed 9.2 km h^{-1}. The distance covered in 12 h by a 51-year-old runner, for whom the curves are presented in Fig. 2d, was 85 km and the average speed 7.0 km h^{-1}.

The heterogeneity of our runners and the small sample size makes it impossible to find a relationship between the intensity of the observed changes in the parameters of the serum denaturation transition and the distance covered. Positive correlation between the average speed and the difference in Cp_m value has been suggested by Pearson's correlation coefficient (r = 0.6; p = 0.1). Statistically significant correlations have been found only between the age and the pre-run values of T_{FM} (r = 0.65; p = 0.04) and HHW (r = 0.82; p = 0.006). Additionally, the age of the participant has appeared negatively correlated with differences between "pre-run" and "post-run" stages in the enthalpy of serum denaturation (r = −0.82; p = 0.006) and in HHW values (r = −0.79; p = 0.01).

4. Discussion

In the present study, DSC method has been used to evaluate the effect of the ultramarathon running on participant's blood serum. DSC allows to generate unique signatures for serum/plasma samples, which can be used for diagnostic purposes and for monitoring the stage of the disease or treatment efficacy (Garbett et al., 2008, 2009; Michnik et al., 2010, 2018; Chagovetz et al., 2011; Todinova et al., 2011, 2012, 2018; Fekecs et al., 2012; Mehdi et al., 2013; Barceló et al., 2015; Kikalishvili et al., 2015; Krumova et al., 2015; Kędra-Królik et al., 2017; Tenchov et al., 2019; Wiegand et al., 2019; Antonova et al., 2020; Dandé et al., 2020;

Table 1
Mean (±SD) parameters of serum DSC transition.

Stage	Tm/°C	Cpm/Jg-1 °C-1	ΔH/J g-1	HHW/°C	TFM/°C
pre-run	70.9 ± 0.9	0.859 ± 0.201	18.55 ± 6.52	23 ± 6	67.0 ± 2.1
post-run	75.8 ± 2.9	1.102 ± 0.226	22.08 ± 5.61	19 ± 3	72.6 ± 2.1

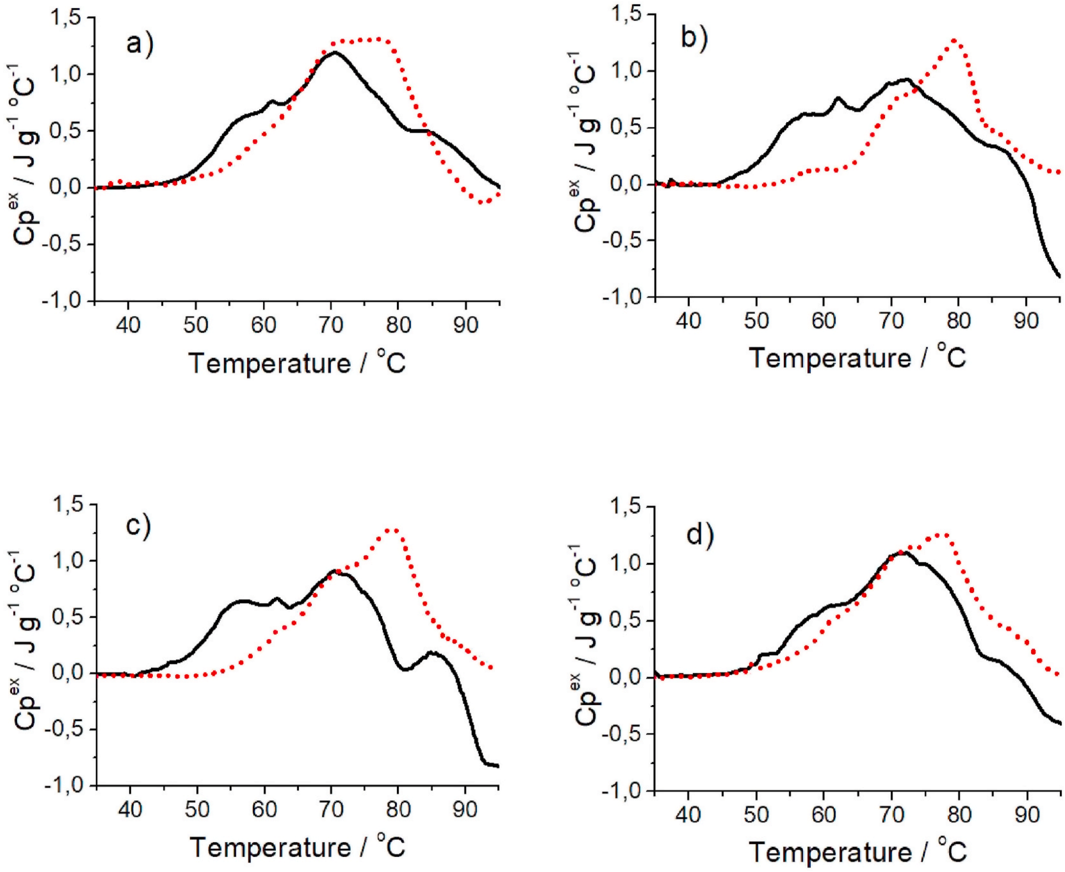

Fig. 2. Serum DSC curves before (solid black line) and after (dot red line) the 12-h run for selected exemples of 4 participants. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Ferencz and Lőrinczy, 2020). Although the complex endothermic DSC transition observed for serum solution arises from the denaturation of the constituent proteins, this method does not rely on identification of the components within serum, rather it relies on the overall DSC pattern generated by a clinical sample of interest. The shape of DSC curves is sensitive to protein structure modifications, protein-protein interactions, and various ligand binding to a protein. The specific biomarkers present in serum in the disease state, or in conditions of disturbed equilibrium of the body for other reasons, can modify the serum thermal denaturation profile. The source of such biomarkers can be biochemical and physiological processes resulting from racing. It has been shown, for example, that endurance exercise evokes transient increases in certain cardiac biomarkers (Scharhag et al., 2008; Żebrowska et al., 2019; Martinez-Navarro et al., 2019). Various blood and urine biomarkers after an ultra-distance race have been compared to their respective baseline levels by Joufroy et al. (2019). We assume that medical consequences of long-term endurance running training and competition should be reflected in the DSC profiles of the sera.

The main findings of this study are significant changes in blood serum DSC profiles of amateur ultramarathon runners immediately after finishing the 12-h running. They are associated with significant changes in the serum proteome and reflect a physiological response to a state of acute stress and volume overload. Ultra-endurance events exert a huge impact on metabolism and energy balance. It seems probable that excessive amounts of intramuscular proteins and metabolic products may be released into the blood stream due to an enhanced metabolism. Hoppel et al. (2019) suggest impaired kidney function in most of the non-elite ultramarathon runners immediately post-race. Ultra-distance races have been shown to increase the accumulation of nitrotirosine and protein carbamyl in serum and urine, thereby suggesting the induction of oxidative stress induced by extreme physical exercise (Levey et al., 2005). Generally, it is accepted that strenuous physical exercise can induce oxidative stress in humans, enhances oxygen consumption and production of reactive oxygen species (Mastaloudis et al., 2001; Imai et al., 2002; Finaud et al., 2006; Lamprecht et al., 2008; Park and Kwak, 2016). However, it is difficult to establish to what extent our results can be explained by oxidative protein modification connected with the stress of exercise.

The changes observed in the shape as well as in parameters of serum denaturation transition after 12 h ultramarathon running are similar but much stronger than these reported by us earlier for elite cross-country skiers after exercise (Michnik et al., 2020b). Those previous studies also stated an increase of T_m, Cp_m, T_{FM} and a decrease of HHW after exercise compared to before exercise stage. These post-exercise effects have been enhanced by sauna treatments (Duch et al., 2020; Michnik et al., 2020a). A shift of the endothermic transition towards higher temperatures was also observed during the aging of serum solutions (Kiełboń et al., 2019). However, during the aging process, the presence of the exothermic peak has been additionally observed in the temperature range preceding the endothermic transition. We did not observe such an effect in the present study.

Despite the fact that the endothermic transition associated with serum denaturation is generated by the whole serum proteome, it seems obvious that the changes observed in DSC profiles of athlete's blood serum should be largely related to the predominant serum protein, albumin. This protein possesses a unique capability to bind, covalently or reversibly, a great number of various endogenous and exogenous compounds. The ability to fluctuate between isomeric forms in aqueous solution could assist in adapting the albumin molecule to bind ligands with a diverse nature with high affinity (Kragh-Hansen, 1990). Both in the blood and in extravascular fluids albumin is susceptible to different oxidative modifications, especially thiol oxidation and carbonylation. Ligand binding and the oxidation of albumin alter its structure. Conformation differences between various forms of albumin (e.g. reduced (human mercaptalbumin, HMA) oxidized (human non-mercaptalbumin, HNA), non-ligated, with bound fatty acids or other ligands, glycated) lead to differences between their DSC profiles. An increasing fraction of albumin carrying different ligands could at least partly explain the upward shift of the endothermic transition observed after the ultramarathon running, since the ligated form of albumin has a higher thermal stability than non-ligated (Michnik et al., 2006; Musante et al., 2006; Garidel et al., 2009; Michnik and Drzazga, 2010; Minami et al., 2014; Bohlooli et al., 2014). The effects of altered carbohydrate metabolism, the regulation of substrate metabolism, protein degradation, an increase in oxygen demand during prolonged exercise affect the state of albumin. Spanidis et al. (2017) examined the oxidation of human serum albumin caused by oxidative stress following an ultramarathon race. They reported that HSA oxidation after ultramarathon race exhibited a great variation between different athletes.

Generally, the thermal transitions observed for serum/plasma of individuals suffering from various diseases have been found significantly shifted towards higher denaturation temperatures when compared with healthy controls. According to Hoffman and Krishnan (2014) study of 1212 active ultramarathon runners, they appear healthier, compared with the general population. However, intense endurance training before the ultramarathon may result in the accumulation of fatigue already at this stage. The mean value of the first moment temperature T_{FM} (about 67 °C) for participants of this study before the run is similar for that found by us earlier for cross country skiers (Michnik et al., 2020a) and slightly higher than the value reported for other group of athletes (Michnik et al., 2013). The pre-run value of T_{FM} has been found significantly correlated with the age of the participant in the current study. A positive value of the Pearson's correlation coefficient ($r = 0.65$) indicates an increase in pre-run T_{FM} value with age. The value of T_{FM} increases significantly to 72.6 °C after the run. Ultramarathon running, being one of the most extreme and strenuous types of exercise, represents a major physical challenge even for elite athletes. The question remains about its safety for amateur runners. Participants of current study were non-elite runners with large interindividual performance differences. Amateurs may be more prone to developing adverse effects of a long-term high-intensity stimulus compared to elite athletes. On the other hand, results of Jastrzębski et al. (2015) study have indicated that organ damage and negative metabolic changes during a 100-km run occur similarly in participants less experienced as well as in well trained runners and are independent of their age. The similar conclusion can be drawn from the comparison of the DSC curves shown in Fig. 2b and c for 63- and 49-years old participants, respectively. In both cases, despite the difference in the age of runners, the changes connected with the 12-h running are large. However, the results of current study suggest smaller differences between "pre-run" and "post-run" stages in the enthalpy of serum denaturation and in the width of the thermal transition with age.

This study has a few limitations worth noting. Firstly, it was conducted on a relatively small group including male subjects only. Therefore, we are not able to discuss possible gender differences. Secondly, the observation time was limited to pre- and post-running. We did not monitor the changes in serum DSC curves during and at the end of the recovery phase. Therefore, we did not observe whether and how quickly the values of the parameters describing the analyzed denaturation transition return to pre-race values. Such information on the recovery time would be valuable, and the DSC method ideally suited to obtain it. However, we did not have serum samples from this period.

5. Conclusions

This study is probably the first to investigate the effects of ultramarathon running using the differential scanning calorimetry method. The results of the DSC study showed that the 12-h running race led to significant changes in the serum proteome of participants. A clear influence of the ultramarathon run on the shape and parameters of the endothermic transition resulting from the denaturation of serum proteins was observed in all participants, who were amateur long-distance runners. The direction of changes was the same, but a large variation in the size of the effect was found due to large interindividual performance differences. The small sample size and the heterogeneity of amateur runners made it impossible to find a relationship between the intensity of the observed changes and the age of the participant and the distance covered.

Author contributions

Conceptualization, A.M. and E.S-K.; Formal analysis, A.M.; Investigation, A.M., A.K., K.D., E.S-K. S.B.; Methodology, A.M. and E.S-K.; Resources. A.M. and E.S-K.; Visualization, A.M., A.K., K.D. S.B.; Writing-original draft, A.M.; Writing-review & editing A.M., E.S-K, A.K., K.D. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Declaration of competing interest

The authors declare no conflict of interest.

References

Antonova, B., Naydenov, E., Koynova, R., Tumangelova-Yuzeir, K., Tenchov, B., 2020. Exothermic transitions in the heat capacity profiles of human cerebrospinal fluid. Eur. Biophys. J. 49, 231–238. https://doi.org/10.1007/s00249-020-01429-w.

Barceló, F., Cerdà, J.J., Gutiérrez, A., Jimenez-Marco, T., Durán, M.A., Novo, A., Ros, T., Sampol, A., Portugal, J., 2015. Characterization of monoclonal gammopathy of undetermined significance by calorimetric analysis of blood serum proteome. PloS One 10 (3), e0120316. https://doi.org/10.1371/journal.pone.0120316.

Bohlooli, M., Moosavi-Movahedi, A.A., Ghaffari-Moghaddam, M., Saboury, A.A., Khajeh, M., Najafi, S., Poormolaie, N., Taghavi, F., Poursasan, N., Sanchooli, M., Esmaeilzadeh, S., Naderi, M., Shahraki, S., 2014. Comparative study of thermal domains analyzing of glycated and non-glycated human serum albumin. Thermochim. Acta 594, 24–30. https://doi.org/10.1016/j.tca.2014.08.034.

Burke, L.M., Castell, L.M., Casa, D.J., Close, G.L., Costa, R.J.S., Melin, A.K., Sygo, J., Desbrow, B., Peeling, P., Witard, O.C., Halson, S.L., Saunders, P.U., Bermon, S., Lis, D.M., Slater, G.J., Stellingwerff, T., 2019. International association of athletics federations consensus statement 2019: nutrition for athletics. Int. J. Sport Nutr. Exerc. Metabol. 29, 73–84. https://doi.org/10.1123/ijsnem.2019-0065.

Chagovetz, A.A., Jensen, R.L., Recht, L., Glantz, M., Chagovetz, A.M., 2011. Preliminary use of differential scanning calorimetry of cerebrospinal fluid for the diagnosis of glioblastoma multiforme. J. Neuro Oncol. 105 (3), 499–506. https://doi.org/10.1007/s11060-011-0630-5.

Coates, A.M., Berard, J.A., King, T.J., Burr, J.F., 2020. Physiological Determinants of Ultramarathon Trail Running Performance. https://doi.org/10.31236/osf.io/y2kdx.

Costa, R.J.S., Gill, S.K., Hankey, J., Wright, A., Marczak, S., 2014. Perturbed energy balance and hydration status in ultra-endurance runners during a 24 h ultramarathon. Br. J. Nutr. 112, 428–437. https://doi.org/10.1017/S0007114514000907.

Dandé, Á., Kocsis, B., Lőrinczy, D., 2020. Thermal analysis of synovial fluids in different stages of osteoarthritis and after bacterial infections. J. Therm. Anal. Calorim. https://doi.org/10.1007/s10973-019-09222-4.

Duch, K., Michnik, A., Pokora, I., Sadowska-Krępa, E., 2020. Modification of blood serum DSC profiles by sauna treatments in cross-country skiers during the exercise cycle. J. Therm. Anal. Calorim. 142, 1927–1932. https://doi.org/10.1007/s10973-020-10162-7.

Duch, K., Michnik, A., Pokora, I., Sadowska-Krępa, E., Kiełboń, A., 2019. Whole-body cryostimulation impact on blood serum thermal denaturation profiles of cross-country skiers. J. Therm. Anal. Calorim. 138, 4505–4511. https://doi.org/10.1007/s10973-019-08766-9.

Fekecs, T., Zapf, I., Ferencz, A., Lőrinczy, D., 2012. Differential scanning calorimetry (DSC) analysis of human plasma in melanoma patients with or without regional lymph node metastases. J. Therm. Anal. Calorim. 108 (1), 149–152. https://doi.org/10.1007/s10973-011-1800-6.

Ferencz, A., Lőrinczy, D., 2020. Surgical stress detection in human blood plasma by DSC. J. Therm. Anal. Calorim. 142, 783–788. https://doi.org/10.1007/s10973-020-09518-w.

Finaud, J., Lac, G., Filaire, E., 2006. Oxidative stress: relationship with exercise and training. Sports Med. 36, 327–358. https://doi.org/10.2165/00007256-200636040-00004.

Garbett, N.C., Mekmaysy, C.S., Helm, C.W., Jenson, A.B., Chaires, J.B., 2009. Differential scanning calorimetry of blood plasma for clinical diagnosis and monitoring. Exp. Mol. Pathol. 86, 186–191. https://doi.org/10.1016/j.yexmp.2008.12.001.

Garbett, N.C., Miller, J.J., Jenson, A.B., Chaires, J.B., 2008. Calorimetry outside the box: a new window into the plasma proteome. Biophys. J. 94, 1377–1383. https://doi.org/10.1529/biophysj.107.119453.

Garidel, P., Hoffmann, C., Blume, A., 2009. A thermodynamic analysis of the binding interaction between polysorbate 20 and 80 with human serum albumins and immunoglobulins: a contribution to understand colloidal protein stabilisation. Biophys. Chem. 143, 70–78. https://doi.org/10.1016/j.bpc.2009.04.004.

Hoffman, M.D., 2016. Injuries and health considerations in ultramarathon runners. Phys. Med. Rehabil. Clin 27 (1), 203–216. https://doi.org/10.1016/j.pmr.2015.08.004.

Hoffman, M.D., Krishnan, E., 2014. Health and exercise-related medical issues among 1,212 ultramarathon runners: baseline findings from the Ultrarunners Longitudinal TRAcking (ULTRA) Study. PloS One 9 (1), e83867. https://doi.org/10.1371/journal.pone.0083867.

Hoffman, M.D., Pasternak, A., Rogers, I.R., Khodaee, M., Hill, J.C., Townes, D.A., Scheer, B.V., Krabak, B.J., Basset, P., Lipman, G.S., 2014. Medical services at ultra-endurance foot races in remote environments: medical issues and consensus guidelines. Sports Med. 44 (8), 1055–1069. https://doi.org/10.1007/s40279-014-0189-3.

Hoffman, M.D., Stellingwerff, T., Costa, R.J.S., 2019. Considerations for ultra-endurance activities: part 2– hydration. Res. Sports Med. 27, 182–194. https://doi.org/10.1080/15438627.2018.1502189.

Hoppel, F., Calabria, E., Pesta, D., Kantner-Rumplmair, W., Gnaiger, E., Burtscher, M., 2019. Physiological and pathophysiological responses to ultramarathon running in non-elite runners. Front. Physiol. 10, 1300. https://doi.org/10.3389/fphys.2019.01300.

Imai, H., Hayashi, T., Negawa, T., Nakamura, K., Tomida, M., Koda, K., Tajima, T., Koda, Y., Suda, K., Era, S., 2002. Strenuous exercise-induced change in redox state of human serum albumin during intensive kendo training. Jpn. J. Physiol. 52, 135e40. https://doi.org/10.2170/jjphysiol.52.135.

Jastrzębski, Z., Zychowska, M., Jastrzębska, M., Prusik, Krzysztof, Prusik, Katarzyna, Kortas, J., Ratkowski, W., Konieczna, A., Radzimiński, Ł., 2015. Changes in blood morphology and chosen biochemical parameters in ultra-marathon runners during a 100-km run in relation to the age and speed of runners. Int. J. Occup. Med. Environ. Health 29, 801–814. https://doi.org/10.13075/ijomeh.1896.00610.

Jouffroy, R., Lebreton, X., Mansencal, N., Anglicheau, D., 2019. Acute kidney injury during an ultra-distance race. PloS One 14 (9), e0222544. https://doi.org/10.1371/journal.pone.0222544.

Kao, W.F., Hou, S.K., Chiu, Y.H., Chou, S.L., Kuo, F.C., Wang, S.H., Chen, J.J., 2015. Effects of 100-km ultramarathon on acute kidney injury. Clin. J. Sport Med. 25 (1), 49–54. https://doi.org/10.1097/JSM.0000000000000116.

Kędra-Królik, K., Chmielewska, I., Michnik, A., Zarzycki, P., 2017. Blood serum calorimetry indicates the chemotherapeutic efficacy in lung cancer treatment. Sci. Rep. 7, 16796 https://doi.org/10.1038/s41598-017-17004-x.

Kiełboń, A., Michnik, A., Polaczek Grelik, K., Duch, K., Sadowska-Krępa, E., 2019. Differential scanning calorimetry of human blood serum exposed in vitro to X-ray radiation. Thermochim. Acta 680, 178358. https://doi.org/10.1016/j.tca.2019.178358.

Kikalishvili, L., Ramishvili, M., Nemsadze, G., Lezhava, T., Khorava, P., Gorgoshidze, M., Kiladze, M., Monaselidze, J., 2015. Thermal stability of blood plasma proteins of breast cancer patients, DSC study. J. Therm. Anal. Calorim. 120 (1), 501–505. https://doi.org/10.1007/s10973-015-4426-2.

Kłapcińska, B., Waśkiewicz, Z., Chrapusta, S.J., Sadowska-Krępa, E., Czuba, M., Langfort, J., 2013. Metabolic responses to a 48-h ultra-marathon run in middle-aged male amateur runners. Eur. J. Appl. Physiol. 113 (11), 2781–2793. https://doi.org/10.1007/s00421-013-2714-8.

Knechtle, B., 2017. Ultramarathon running: medical issues. In: Feletti, F. (Ed.), Extreme Sports Medicine. Springer, Cham. https://doi.org/10.1007/978-3-319-28265-7_13.

Knechtle, B., Nikolaidis, P., 2015. Ultra-marathon running. Dansk Sportsmedicin 19 (4), 6–10.

Knechtle, B., Nikolaidis, P.T., 2018. Physiology and pathophysiology in ultra-marathon running. Front. Physiol. 9, 634. https://doi.org/10.3389/fphys.2018.00634.

Knechtle, B., Rüst, C.A., Rosemann, T., Lepers, R., 2012. Age-related changes in 100-km ultra-marathon running performance. Age (Omaha). 34 (4), 1033–1045. https://doi.org/10.1007/s11357-011-9290-9.

Knechtle, B., Tanda, G., 2015. Effects of training and anthropometric factors on marathon and 100 km ultramarathon race performance. Open Access J. Sports Med. 129 https://doi.org/10.2147/oajsm.s80637.

Kragh-Hansen, U., 1990. Structure and ligand binding properties of human serum albumin. Dan. Med. Bull. 37 (1), 57–84.

Krumova, S., Todinova, S., Danailova, A., Petkova, V., Dimitrova, K., Gartcheva, L., Taneva, S.G., 2015. Calorimetric features of IgM gammopathies. Implication for patient's diagnosis and monitoring. Thermochim. Acta 615, 23–29. https://doi.org/10.1016/j.tca.2015.07.002.

Lamprecht, M., Greilberger, J.F., Schwaberger, G., Hofmann, P., Oettl, K., 2008. Single bouts of exercise affect albumin redox state and carbonyl groups on plasma protein of trained men in a workload-dependent manner. J. Appl. Physiol. 104, 1611–1617. https://doi.org/10.1152/japplphysiol.01325.2007.

Lehto, N., 2016. Effects of age on marathon finishing time among male amateur runners in Stockholm Marathon 1979–2014. J. Sport Health Sci. 5 (3), 349–354. https://doi.org/10.1016/j.jshs.2015.01.008.

Levey, A.S., Eckardt, K.U., Tsukamoto, Y., Levin, A., Coresh, J., Rossert, J., De Zeeuw, D., Hostetter, T.H., Lameire, N., Eknoyan, G., Willis, K., 2005. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: improving Global Outcomes (KDIGO)z. Kidney Int. 67 (6), 2089–2100. https://doi.org/10.1111/j.1523-1755.2005.00365.x.

Martínez-Navarro, I., Sanchez-Gómez, J.M., Aparicio, I., Priego-Quesada, J.I., Pérez-Soriano, P., Collado, E., Hernando, B., Hernando, C., 2020. Effect of mountain ultramarathon distance competition on biochemical variables, respiratory and lower-limb fatigue. PloS One 15 (9), e0238846. https://doi.org/10.1371/journal.pone.0238846.

Martínez-Navarro, I., Sánchez-Gómez, J.M., Collado-Boira, E.J., Hernando, B., Panizo, N., Hernando, C., 2019. Cardiac damage biomarkers and heart rate variability following a 118-km mountain race: relationship with performance and recovery. J. Sports Sci. Med. 18, 615–622.

Mastaloudis, A., Leonard, S.W., Traber, M.G., 2001. Oxidative stress in athletes during extreme endurance exercise. Free Radic. Biol. Med. 31, 911–922. https://doi.org/10.1016/S0891-5849(01)00667-0.

Mehdi, M., Fekecs, T., Zapf, I., Ferencz, A., Lőrinczy, D., 2013. Differential scanning calorimetry (DSC) analysis of human plasma in different psoriasis stages. J. Therm. Anal. Calorim. 111 (3), 1801–1804. https://doi.org/10.1007/s10973-012-2468-2.

Michnik, A., Drzazga, Z., 2010. Thermal denaturation of mixtures of human serum proteins: DSC study. In: J. Therm. Anal. Calorim., 101, pp. 513–518. https://doi.org/10.1007/s10973-010-0826-5.

Michnik, A., Drzazga, Z., Michalik, K., Barczyk, A., Santura, I., Sozańska, E., Pierzchała, W., 2010. Differential scanning calorimetry study of blood serum in chronic obstructive pulmonary disease. J. Therm. Anal. Calorim. 102, 57–60. https://doi.org/10.1007/s10973-009-0602-6.

Michnik, A., Drzazga, Z., Poprzęcki, S., Czuba, M., Kempa, K., Sadowska-Krępa, E., 2013. DSC serum profiles of sportsmen. In: J. Therm. Anal. Calorim., 113, pp. 365–370. https://doi.org/10.1007/s10973-013-3130-3.

Michnik, A., Drzazga, Z., Schisler, I., Poprzęcki, S., Czuba, M., 2018a. Diversity in athlete's response to strength effort in normobaric hypoxia: serum DSC study. J. Therm. Anal. Calorim. 134, 633–641. https://doi.org/10.1007/s10973-018-7067-4.

Michnik, A., Duch, K., Pokora, I., Sadowska Krępa, E., 2020a. Differences in cryostimulation and sauna effects on post-exercise changes in blood serum of athletes. Compl. Ther. Med. 51, 102453. https://doi.org/10.1016/j.ctim.2020.102453.

Michnik, A., Michalik, K., Kluczewska, A., Drzazga, Z., 2006. Comparative DSC study of human and bovine serum albumin. J. Therm. Anal. Calorim. 84, 113–117. https://doi.org/10.1007/s10973-005-7170-1.

Michnik, A., Pokora, I., Duch, K., Sadowska-Krępa, E., 2020b. Differential scanning calorimetry reveals that whole-body cryostimulation in cross-country skiers can modify their response to physical effort. J. Therm. Anal. Calorim. https://doi.org/10.1007/s10973-020-09285-8.

Michnik, A., Sadowska-Krępa, E., Cholewa, J., Schisler, I., Kiełboń, A., Drzazga, Z., 2018b. Differential scanning calorimetry study of early and advanced stages in Parkinson's disease using human blood serum. Thermochim. Acta 662, 64–68. https://doi.org/10.1016/j.tca.2018.02.014.

Minami, T., Terada, T., Takahashi, T., Arikawa, H., Matsuyama, Y., Kizaki, K., Era, S., 2014. Comparative studies on the heterogeneity of plasma-derived and recombinant human albumins in laboratory use. Int. J. Biol. Macromol. 69, 79–87. https://doi.org/10.1016/j.ijbiomac.2014.05.010.

Musante, L., Bruschi, M., Candiano, G., Petretto, A., Dimasi, N., Boccio, P. Del, Urbani, A., Rialdi, G., Ghiggeri, G.M., 2006. Characterization of oxidation end product of plasma albumin "in vivo. Biochem. Biophys. Res. Commun. 349, 668–673. https://doi.org/10.1016/j.bbrc.2006.08.079.

Nikolaidis, P.T., Knechtle, B., 2020. Performance in 100-km ultramarathoners-at which age, it reaches its peak? J. Strength Cond. Res. 34 (5), 1409–1415. https://doi.org/10.1519/JSC.0000000000002539.

Park, S.-Y., Kwak, Y.-S., 2016. Impact of aerobic and anaerobic exercise training on oxidative stress and antioxidant defense in athletes. J. Exerc. Rehabil. 12 (2), 113–118. https://doi.org/10.12965/jer.1632598.299.

Rodrigues Júnior, J.F.C., Mckenna, Z., Amorim, F.T., Da Costa Sena, A.F., Mendes, T.T., Veneroso, C.E., Torres Cabido, C.E., Sevilio De Oliveira Júnior, M.N., 2020. Thermoregulatory and metabolic responses to a half-marathon run in hot, humid conditions. J. Therm. Biol. 93, 102734. https://doi.org/10.1016/j.jtherbio.2020.102734.

Scharhag, J., George, K., Shave, R., Urhausen, A., Kindermann, W., 2008. Exercise-associated increases in cardiac biomarkers. Med. Sci. Sports Exerc. 40, 1408–1415. https://doi.org/10.1249/MSS.0b013e318172cf22.

Scheer, V., Basset, P., Giovanelli, N., Vernillo, G., Millet, G.P., Costa, R.J.S., 2020. Defining off-road running: a position statement from the ultra sports science foundation. Int. J. Sports Med. 41, 275–284. https://doi.org/10.1055/a-1096-0980.

Spanidis, Y., Priftis, A., Stagos, D., Stravodimos, G.A., Leonidas, D.D., Spandidos, D.A., Tsatsakis, A.M., Kouretas, D., 2017. Oxidation of human serum albumin exhibits inter-individual variability after an ultra- marathon mountain race. Exp. Ther. Med. 13, 2382–2390. https://doi.org/10.3892/etm.2017.4268.

Spittler, J., Oberle, L., 2019. Current trends in ultramarathon running. Curr. Sports Med. Rep. 18 (11), 387–393. https://doi.org/10.1249/JSR.0000000000000654.

Tenchov, B., Koynova, R., Antonova, B., Zaharinova, S., Abarova, S., Tsonchev, Z., Komsa-Penkova, R., Momchilova, A., 2019. Blood plasma thermal behavior and protein oxidation as indicators of multiple sclerosis clinical status and plasma exchange therapy progression. Thermochim. Acta 671, 193–199. https://doi.org/10.1016/j.tca.2018.12.001.

Tiller, N.B., 2019. Pulmonary and respiratory muscle function in response to marathon and ultra-marathon running: a review. Sports Med. 49 (7), 1031–1041. https://doi.org/10.1007/s40279-019-01105-w.

Todinova, S., Krumova, S., Danailova, A., Petkova, V., Guenova, M., Mihaylov, G., Gartcheva, L., Taneva, S.G., 2018. Calorimetric markers for monitoring of multiple myeloma and Waldenström's macroglobulinemia patients. Eur. Biophys. J. 83, 7992–7998. https://doi.org/10.1007/s00249-018-1277-3.

Todinova, S., Krumova, S., Gartcheva, L., Robeerst, C., Taneva, S.G., 2011. Microcalorimetry of blood serum proteome: a modified interaction network in the multiple myeloma case. Anal. Chem. https://doi.org/10.1021/ac202055m.

Todinova, S., Krumova, S., Kurtev, P., Dimitrov, V., Djongov, L., Dudunkov, Z., Taneva, S.G., 2012. Calorimetry-based profiling of blood plasma from colorectal cancer patients. Biochim. Biophys. Acta Gen. Subj. 1820, 1879–1885. https://doi.org/10.1016/j.bbagen.2012.08.001.

Waśkiewicz, Z., Kłapcińska, B., Sadowska-Krępa, E., Czuba, M., Kempa, K., Kimsa, E., Gerasimuk, D., 2012. Acute metabolic responses to a 24-h ultra-marathon race in male amateur runners. Eur. J. Appl. Physiol. 112 (5), 1679–1688. https://doi.org/10.1007/s00421-011-2135-5.

Waśkiewicz, Z., Nikolaidis, P.T., Chalabaev, A., Rosemann, T., Knechtle, B., 2019. Motivation in ultra- marathon runners. Psychol. Res. Behav. Manag. 12, 31–37. https://doi.org/10.2147/PRBM.S189061.

Wiegand, N., Bűcs, G., Dandé, Á., Lőrinczy, D., 2019. Investigation of protein content of synovial fluids with DSC in different arthritides. J. Therm. Anal. Calorim. 138, 4497–4503. https://doi.org/10.1007/s10973- 019-08151-6.

Żebrowska, A., Waśkiewicz, Z., Nikolaidis, P.T., Mikołajczyk, R., Kawecki, D., Rosemann, T., Knechtle, B., 2019. Acute responses of novel cardiac biomarkers to a 24-h ultra-marathon. J. Clin. Med. 8, 57. https://doi.org/10.3390/jcm8010057.