

SECTION – FUNDAMENTAL AND APPLIED KINESIOLOGY

DOI:

IMPACT OF SYSTEMIC CRYOTHERAPY ON THE RHEOLOGICAL PROPERTIES OF THE BLOOD IN HEALTHY YOUNG MALES

Authors' contribution:

- A. Study design/planning
- B. Data collection/entry
- C. Data analysis/statistics
- D. Data interpretation
- E. Preparation of manuscript
- F. Literature analysis/search
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Key words: systemic cryotherapy, blood rheology

Abstract

Study aim: The aim of the study was to examine the influence of systemic cryotherapy on the rheological properties of the blood.

Study material: The study groups consisted of 10 healthy males, aged 23-24 (23.4+/-0.52), who underwent systemic cryotherapy treatments (3 min treatment time, -120°C chamber temperature). In order to analyze the rheological parameters of the blood, venous blood samples were drawn from the study participants three times. The first analysis took place two months before cryotherapy, the second on the day of beginning treatments, and the third was performed after a series of 10 treatments.

Results: In the study group, the mean values of RBC and HCT following the series of 10 treatments were significantly higher after cryotherapy in comparison to the measurements taken two months before as well as on the day of beginning treatments. However, comparing the group two months before cryotherapy with the group on the day of its commencement, a decrease in HCT and MCV levels was found. Analyzing the mean concentrations of HGB, MCHC, MCH, MCV in the young males, statistically significant reduction after the series of 10 treatments was found compared to the values obtained two months before testing and on the day of beginning treatment. However, comparing the group two months before and on the day of beginning cryotherapy treatments, an increase in the MCHC index was found. The average values of WBC and PLT after a series of 10 treatments were higher compared with the values obtained two months before the study. Increased levels of WBC were found comparing the group two months before to the group on the day of beginning cryotherapy treatments. PLT values were higher after a series of 10 treatments compared with the values obtained on the day of beginning treatments. Analyzing the number of EI at shear stress from 0.30 to 59.97 [Pa], a decrease in $SS_{1/2}/EI_{max}$ i $SS_{1/2}/EI_{max}$ values was found before and after systemic cryotherapy treatment. The lower the values of $SS_{1/2}/EI_{max}$ and $SS_{1/2}/EI_{max}$, the greater the deformability of erythrocytes.

Conclusions: Systemic cryotherapy does not cause any side effects in healthy young males and its regular usage positively affects the rheological properties of the blood.

Introduction

Systemic cryotherapy is used to induce physiological, organ and systemic defensive reactions, which are beneficial and effective in maintaining or restoring ho-

meostasis of the human body [1]. This method is used to alleviate pain and inflammation in degenerative diseases as well as neurological and rheumatoid disorders. The recommended therapy consists of combining cold with exercise, which creates favourable conditions for the im-



provement of the cardiovascular system. It can also lead to decreased skeletal muscle tension, reduced edema and increased muscle strength. It improves metabolism and well-being, accelerates regeneration and repair processes and improves mobility of the treated joints as well [2,3].

The usage of systemic cryotherapy is one of the ways to reduce pain threshold or its abolition. Systemic use of extremely low temperatures results in increased exercise tolerance and immunity of the body, and has a positive effect on the psyche, causing decrease of fear, anxiety and hyperactivity; improving perception and concentration and creating better resistance of central nervous system to fatigue [4,5].

The occurrence of erythrocyte deformability plays an important role in the flow of blood cells through the capillaries having a diameter up to two times smaller than the cells themselves. Normal erythrocytes are capable of deformation under stress primarily due to the fact that they have no nucleus, the cytoplasm has a relatively low viscosity, the cell membrane has advantageous visco-elastic properties, and their appropriate shape provides a high ratio of free surface to volume [6, 7]. Changes in the shape of the cell depend on the quality of the spectrin-actin network in conjunction with calcium ions and ATP [8, 9]. The reason for the decline of this capacity is mainly their age, mechanical damage and disease factors.

Changes in shape do not cause changes in volume or surface, and once transferred into larger vessels, the blood cell returns to its previous shape within a short period of time. This does not affect the structure or function of the erythrocyte [10]. The cells during the flow through the vessels may take various forms: twisted, folded in half, similar to a parachute or torpedo [11].

The rheological phenomenon is that even with a hematocrit higher than 80%, the blood remains fluid in contrast to the suspension of rigid molecules, which take the form of a solid consistency at a concentration of about 65%. This is caused by the liquidity of blood cells which can be treated as liquid droplets surrounded by a membrane [12, 13]. The phenomenon of spontaneous aggregation of red blood cells in whole blood, namely the formation of three dimensional erythrocyte structure, is a reversible physiological phenomenon that plays a significant role in blood flow at low shear rates and significantly affects the increase in blood viscosity [14, 15].

The aim of this study was to assess the effects of systemic cryotherapy on the rheological parameters of the blood in young healthy males.

Study material and methods

The study group consisted of 10 healthy males, aged 23–24 (23.4+/-0.52), who underwent systemic cryotherapy treatments. In order to analyze morphological

and rheological parameters of the blood, venous blood samples were drawn from the participants of the study three times. The first study was held on 6th March, 2014 (two months before cryotherapy), the second on the day of beginning treatments on 5th May, 2014 and the third test was conducted after a series of 10 treatments on 19th May, 2014.

The parameters obtained in the cryo-chamber:

- aerial temperature: -60°C
- chamber temperature: -120°C

The time of a single treatment for the group of males was 1.5 min (1st treatment), 3 min (2–10th treatments). 3 ml of blood were drawn from the vein inside the elbow from the participants on an empty stomach in the morning, into EDTA tubes. Blood samples were drawn by a qualified nurse under medical supervision, in accordance with applicable standards of the Pathology of Locomotion Laboratory at the University School of Physical Education in Krakow, where rheological and morphological parameters of the blood were determined. The study was approved by the Bioethics Committee at the Regional Medical Chamber in Krakow.

Determination of elongation index

Erythrocyte deformability was tested using the LORCA analyzer (Laser-assisted Optical Rotational Cell Analyser RR Mechatronics, The Netherlands). The results were obtained as the index of elongation and aggregation according to the Hardeman method [16, 17]. Tests using the above apparatus were conducted within 30 minutes after blood collection, at 37 °C and according to standard protocol.

Blood for the determination of the elongation index was collected in an amount of 25 µl to 5 ml 0.14 mM PVP (*polyvinylpyrrolidone*, M = 360,000, Sigma, viscosity at 37°C above 31mPa) and dissolved in phosphate buffered saline (PBS). The test sample was placed in a measuring chamber between two rotating concentric cylinders. The laser light passing through the thin layer of red blood cells suspended in PBS underwent deflection, giving a diffraction pattern on the projection screen. The diffraction pattern was recorded with a video camera and then transferred to a computer and is dependent on the value of shear stress acting on the blood cell during the rotation of the cylinder. However, as the tension rises, the shape of the diffraction pattern shifts from a circle to an ellipse with an increasing ratio of the long axis “a” to the short “b”. The index was calculated by:

$$EI = \frac{a - b}{a + b}$$



The results of the elongation index (EI) were given in the range of 0.30 to 59.97 of the shear stress measured in Pascals. The elongation index is a measure of the amount of deformation of red blood cells during their movement in the measuring chamber [16, 17].

Determination of aggregation index

Before the actual test, the blood sample was subjected to oxygenation by incubation and mixing with carbogen within 15 minutes of collection. Blood in the amount of 1.5 ml was put into the measuring chamber of the LORCA analyzer. The computer-controlled cylinder was rotated within 120 sec, and the shear stress was $> 400 \text{ s}^{-1}$. After 10 seconds, rotation of the cylinder was stopped and aggregation of erythrocytes began, which was measured by change in the intensity of the laser beam passing through (*backscattering*), until the highest value was reached (between 0.5 and 2.0 s). From this time on (fragment between 2.0 sec and 1 min or longer), the device subjected the aggregates of the red blood cells to various rates of shear stress from 6 to 700 s^{-1} . The result of computer analysis is the curve presenting the relationship of the scattered light intensity with time (for a given shear rate), i.e. selectogram [24, 25].

The following parameters determining erythrocyte aggregation kinetics were assessed:

- AI [%] – aggregation index
A – area above selectogram curve
B – area below selectogram curve

$$AI = \frac{A}{A+B} \times 100\%$$

Also analyzed were:

- AMP [au] – total extent of aggregation
- $T_{1/2}$ [s] – half time kinetics of aggregation

Morphological blood test

Measurements were taken using the ABX MICROS 60 (USA) haematology analyzer. The study used 10 ml of whole blood drawn into K3EDTA.

Determined parameters:

1. Red blood cell count – RBC [$10^{12}/\text{L}$]
2. Hematocrit – Hct [L/L]
3. Haemoglobin – Hgb [g/L]
4. Mean corpuscular hemoglobin index – MCH [fmol]
5. Mean corpuscular volume index – MCV [fL]
6. Mean corpuscular hemoglobin concentration – MCHC [mmol/L]
7. White blood cell count – WBC [$10^9/\text{L}$]
8. Platelet count: PLT [$10^9/\text{L}$]

Measurement of plasma viscosity

After centrifugation of cellular blood components, the obtained 0.5 ml of plasma was put into the measurement capillary of the viscometer. The viscosity of the blood plasma was determined in the viscometer (type D-52159 Roetgen, Myrenne Co., Germany).

Determination of plasma fibrinogen

50 μL of plasma was used for the study. Determination was performed using the Bio-Ksel, Chrom – 7 camera.

Statistical analysis

Data was presented by the mean values and standard deviation ($\bar{x} \pm \text{SD}$). Normality of distribution was verified using the Shapiro-Wilk test. The differences between the resulting measurements were analyzed by one-dimensional analysis of variance (ANOVA) for systems with reproducible measurements. Sphericity was assessed using the Mauchly's test. In the case that the sphericity assumption was not met, the multi-dimensional test was used (Wilks' lambda). When the ANOVA parametric assumptions were not met, differences between the measurements were calculated using the Friedman ANOVA test. Appropriate post-hoc tests were applied to evaluate the differences between particular measurements.

$\text{SS}_{1/2}$ and EI_{max} values were calculated by matching elongation curves with the Lineweaver-Burke model (1), using the non-linear matching algorithm in the GraphPad Prism 6.05 program (GraphPad Software Inc., La Jolla, CA). The method has been described in detail by Baskurt et al. [18, 19, 20]. The quality of matching the curves was evaluated by the R^2 coefficient of determination.

$$\frac{1}{\text{EI}} = \frac{\text{SS}_{1/2}}{\text{EI}_{\text{max}}} \times \frac{1}{\text{SS}} + \frac{1}{\text{EI}_{\text{max}}} \quad (1)$$

In analyzes, the following level of significance $\alpha = 0.005$ was assumed. Analyses were performed using Statistica 10 (StatSoft®, USA).

Results

The mean values of RBC and HCT after a series of 10 treatments were significantly higher following systemic cryotherapy in relation to the measurements taken two months before and on the day of beginning treatment. In contrast, comparing the group two months before the date of beginning the treatments, a decrease in the levels of HCT and MCV can be found (Tab. 1).



Table 1. Mean values \pm standard deviation of blood morphology indicators in males before and after systemic cryotherapy.

Cryotherapy	Measurement 1 (n = 10)	Measurement 2 (n = 10)	Measurement 3 (n = 10)	p	p 1/2	p 2/3	p 1/3
RBC($10^{12}/L$)	4.46 \pm 0.29	4.36 \pm 0.28	5.25 \pm 0.42	0.0000	0.5934	0.0001	0.0001
Hct [L/L]	41.32 \pm 1.19	39.79 \pm 2.11	45.89 \pm 2.13	0.0000	0.0190	0.0000	0.0000
HGB [g/dl]	15.92 \pm 0.57	15.94 \pm 0.53	15.28 \pm 0.85	0.0136	0.8946	0.0003	0.0004
MCH [fmol]	35.76 \pm 2.53	36.67 \pm 2.17	28.60 \pm 1.56	0.0000	0.0906	0.0001	0.0001
MCV (fL)	92.90 \pm 4.41	91.30 \pm 4.16	86.00 \pm 3.86	0.0000	0.0007	0.0000	0.0000
MCHC [mmol/L]	38.53 \pm 0.92	40.11 \pm 1.70	33.30 \pm 0.57	0.0000	0.0084	0.0001	0.0001
WBC [$10^9/L$]	5.32 \pm 1.05	6.12 \pm 1.44	6.08 \pm 1.07	0.0430	0.0255	0.9044	0.0327
PLT [$10^9/L$]	206.80 \pm 37.51	191.70 \pm 23.87	233.60 \pm 31.18	0.0006	0.1037	0.0002	0.0070
FIBR [g/L]	3.47 \pm 0.98	3.84 \pm 0.67	3.38 \pm 0.80	0.3201	–	–	–
plasma viscosity [mPa x s]	1.23 \pm 0.02	1.26 \pm 0.06	1.24 \pm 0.04	0.0921	–	–	–
AI [%]	57.327 \pm 5.8581	58.635 \pm 3.1206	54.383 \pm 5.9436	0.0477	0.7031	0.0432	0.1920
AMP [au]	18.631 \pm 2.9925	18.626 \pm 2.4931	19.284 \pm 2.8845	0.5439	–	–	–
T $\frac{1}{2}$ [s]	2.884 \pm 0.8233	18.626 \pm 2.4931	19.284 \pm 2.8845	0.0648	–	–	–

Description: measurement 1 – two months before cryotherapy; measurement 2 – day of beginning cryotherapy; measurement 3 – after series of 10 treatments

Analyzing the mean concentrations of HGB, MCHC, MCH, MCV in the young males, statistically significant reduction after the series of 10 treatments was found compared to the values obtained two months before testing and on the day of beginning treatment. However, comparing the group two months before and on the day of beginning cryotherapy treatments, an increase in the MCHC index was found (Tab. 1)

The average values of WBC and PLT after a series of ten treatments were higher compared with the values obtained 2 months before treatment. Elevated levels of WBC have been found when comparing the group two months before and on the day of beginning cryotherapy treatments. PLT values after a series of 10 treatments were higher than the values obtained on the day of starting treatment (Tab. 1).

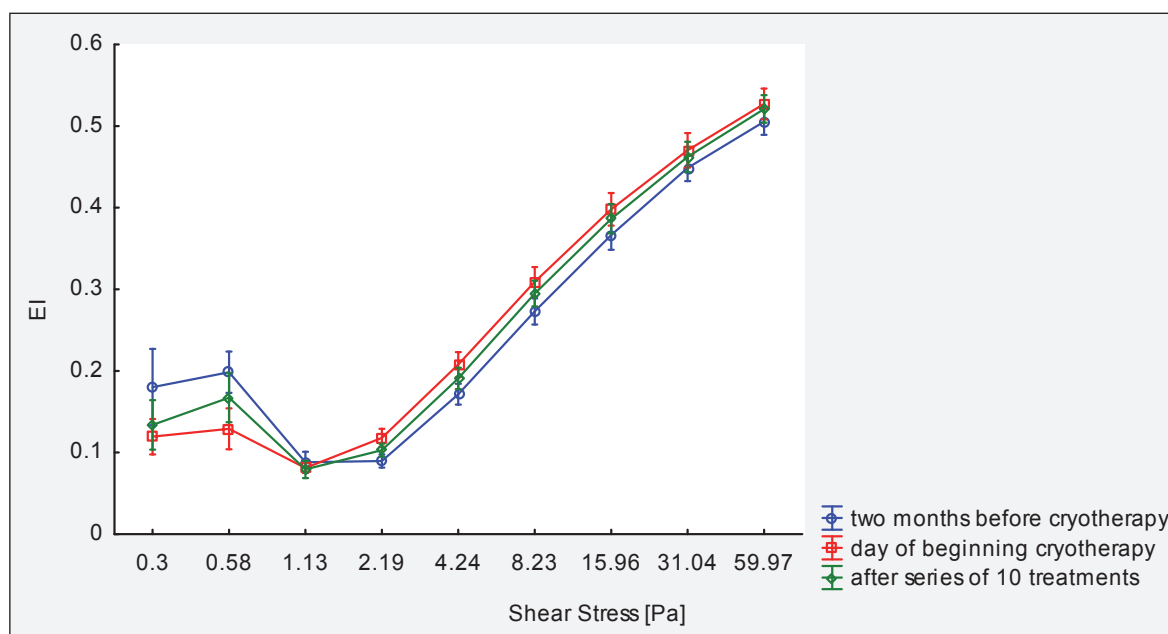
Fig. 1. Comparison of EI values at shear stress from 0.30 to 59.97 Pa in people undergoing systemic cryotherapy before and after a series of treatments.**Fig. 1.** Diagram of mean EI values for males before and after systemic cryotherapy

Table 2. Changes in $SS_{1/2}$, EI_{max} and $SS_{1/2}/EI_{max}$ ratio, presented as mean values \pm standard deviation ($\bar{x} \pm SD$), calculated by matching elongation curves with the Lineweaver-Burke model, two months before cryotherapy, at the beginning of treatment and after a series of 10 treatments.

	n	Measurement 1 (n=10)	Measurement 2 (n=10)	Measurement 3 (n=10)	p	p 1/2	p 2/3	p 1/3
$SS_{1/2}$ [Pa]	10	9.92 \pm 0.89	7.97 \pm 0.75	8.93 \pm 0.60	0.0000	0.0001	0.0002	0.0002
EI_{max}	10	0.59 \pm 0.03	0.60 \pm 0.03	0.60 \pm 0.03	0.3716	0.4358	0.6247	0.2118
$SS_{1/2}/EI_{max}$ [Pa]	10	16.83 \pm 1.82	13.45 \pm 1.69	14.97 \pm 1.50	0.0000	0.0001	0.0002	0.0001

Description: measurement 1 – two months before cryotherapy; measurement 2 – day of beginning cryotherapy; measurement 3 – after series of 10 treatments

Analyzing the number of EI at shear stress from 0.30 to 59.97 [Pa], a decrease in $SS_{1/2}$, EI_{max} and $SS_{1/2}/EI_{max}$ values was found before and after systemic cryotherapy (Tab. 2, Fig. 1). The lower the $SS_{1/2}$, EI_{max} and $SS_{1/2}/EI_{max}$ values, the greater the deformability of erythrocytes.

Considering the values of AI [%] in males before and after systemic cryotherapy, no statistically significant differences were found. In the study group, after a series of 10 treatments, reduced values of AI [%] were noted in the males following cryotherapy in relation to the measurements taken on the first day of treatments (Tab. 1).

There were no statistically significant differences in values of $T_{1/2}$ [s], AMP [au], blood plasma viscosity or fibrinogen in the males before or after systemic cryotherapy (Tab. 1).

Discussion

The research presented in this paper is intended to show changes in the rheological properties of blood in young healthy males who underwent a series of 10 systemic cryotherapy treatments at $-120^{\circ}C$.

A review of literature indicates a lack of detailed data on the effects of systemic cryotherapy on rheological properties of the blood.

The results of the research carried out so far (very little) are difficult to interpret or compare because of differences in their research protocols. Łubkowska and Szygula (2010) showed that the number of cryotherapy sessions (3 min at $-130^{\circ}C$) has significant impact on changes in morphological indices [21]. Moreover, it is very often the case that changes observed following cryotherapy are interpreted in relation to the results obtained after applying near $0^{\circ}C$ temperature, such as immersion or bathing in cold water. Kępińska et al. (2013) described assessment of the impact of a single systemic cryotherapy treatment (3 min at $120^{\circ}C$) on morphological and rheological properties of the blood in healthy males. The study involved five healthy men (aged 20–25). To analyze the morphological and rheological properties of the blood, blood was drawn immediately prior to treatment, approx. 20 - 30 minutes and 24 hours after treatment. Morphological (RBC, Hb, Ht, MCHC) and rhe-

ological properties (elongation index (EI), aggregation index (AI)) were determined. The authors indicated that a single systemic cryotherapy treatment does not result in any statistically significant changes in the morphological or rheological properties of the blood in healthy individuals [22].

The aim of a different study by Kepinska et al. (2014) was to evaluate the effect of a series of cryotherapy treatments (3 min at $120^{\circ}C$) on the morphological and rheological properties of the blood in healthy males. This time, the study involved 10 healthy males, aged 22.1 ± 2.16 . 24 cryotherapy treatments were performed (3 times a week, every other day). There was a statistically significant decrease in mean corpuscular haemoglobin concentration and an increase in the average size of the platelets. Again, however, there were no statistically significant changes in the rheological properties of the blood [23].

After a few days of stimulation by cryogenic temperatures, an increase in the level of haemoglobin, platelet count and creatinine concentration as well as severity of glycaemia was observed [24, 25]. Some reports indicate a decrease in erythrocytes [26, 27, 28, 29, 30] and an increase in leukocyte number [21, 31], while others declare no change in the number of erythrocytes and/or white blood cells, most likely due to the low number of sessions [24, 26, 28, 29, 32]. A decrease in leukocytes and erythrocytes in healthy individuals after a series of treatments was observed by Blatteis (1998) [33]. However, Banfi, et al. (2008) showed a decrease in haemoglobin concentration with a simultaneous increase in the average mass of the haemoglobin molecule and the mean haemoglobin concentration in the erythrocyte after completion of treatments (30 seconds at $60^{\circ}C$ and 2 min at $-110^{\circ}C$) [26].

On the basis of our own research, we found that after a series of 10 treatments, the mean values of erythrocytes, hematocrit and platelet count, were significantly higher compared to measurements taken two months before and on the day of beginning the cryotherapy treatment. However, a significant decrease between these groups was found in the following blood morphology indicators: haemoglobin, MCHC, MCH and MCV. Based on these studies, it can be concluded that systemic cryotherapy is a powerful stimulus and inducement of

the earlier mentioned symptoms can be explained as acclimatization to the prevailing conditions.

The 2-month gap in systemic therapy application affected the following changes in the studied morphological indicators; there was an increase in leukocytes and MCHC, and a decrease in MCV and hematocrit. We know that hematocrit may undergo a change of 6–8% depending on the state of the organism's hydration. In the research, HCT decreased by 3.7%, which is a small difference between with the values obtained two months before testing and on the starting date of treatment.

On the basis of the study, an increase in red blood cell deformability at 0.30 Pa and 0.58 Pa shear stress could be observed before and after systemic cryotherapy treatment. The lower the $SS_{1/2} \cdot EI_{max}$ i $SS_{1/2}/EI_{max}$ values, the greater the deformability of erythrocytes. Statistically low values indicate a high deformability of erythrocytes, both before and after systemic cryotherapy, with a decrease in the index of aggregation. However, no significant changes in the level of half time kinetics of aggregation ($T_{1/2}$), the total extent of aggregation (AMP), the viscosity of the blood plasma or fibrinogen were noted. The usage of systemic cryotherapy acts as a stimulus by activating the adaptive changes in the deformability of erythrocytes in a constricted blood vessel.

This form of treatment leads to increased constriction of the spleen and ejection of the erythrocytes stored in it into the bloodstream, resulting in differences in their formability. Each day, 200-250 billion erythrocytes decompose under physiological conditions, and systemic cryostimulation probably forces a faster breakdown of red blood cells. This phenomenon requires further study.

Also observed was also a significant increase in EI values at 1,13 [Pa] shear stress which proves that regular immersion in cold water has an effect on the increase in erythrocyte deformability in a constricted vascular system. In our study, even though "a different kind of cold" was applied, the same relationship could be observed [34]. In subsequent studies, in order to confirm earlier reports, Teległów et al. (2014) compared a group of winter swimmers with a group undergoing systemic cryotherapy. Nonetheless, the authors found that these interventions had no effect on the parameters of aggregation [35].

As suspected, the conducted studies showed changes in the rheological properties of the blood in young healthy males undergoing systemic cryotherapy. However, these studies require expansion to become acquainted with the body's response under these conditions.

References

- [1] Brojek W: *Krioterapia – co należy wyjaśnić*. Acta Bio-Opt Inform Med. 2005; 12: 68–70.
- [2] Korzonek-Szlacheta I, Wielkoszyński T, Stanek A, Świętochowska E, Karpe J, Sieroń A: *Wpływ krioterapii ogólnoustrojowej na stężenie wybranych hormonów u zawodników wyczynowo uprawiających piłkę nożną*. Endokrynol Pol. 2007; 58: (1): 27–32.
- [3] Stanek A, Cieślak G, Sieroń A: *Zastosowanie krioterapii w medycynie sportowej*. Rehabilitacja w Praktyce. 2008; 3: (2): 34–35.
- [4] Swenson C, Sward L, Karlsson J: *Cryotherapy in sports medicine*. Scand J Med Sci Spor. 1997; 193–200.
- [5] Biały D, Zimmer K, Zagrobelny Z: *Krioterapia ogólnoustrojowa w sporcie*. Med Sport. 1999; 15: (94): 21–24.
- [6] Słowińska L, Monkos K: *Kliniczne zastosowania laserowo-optycznego rotacyjnego analizatora krwinek czerwonych LORCA*, Annal Acad Med Siles. 2010; 64: (3–4) 42–47.
- [7] Bareford D, Stone PCV, Caldwell NM, Meiselman HJ, Stuart J: *Comparison of instruments for measurement of erythrocyte deformability*, Clin Hemorheol. 1985; 5: 311–322.
- [8] Konstantinova E, Tolstaya T, Prishchep S, Milutin A, Miornova E, Ivanova L: *Plasma lipid levels, blood rheology, platelet aggregation, microcirculation state and oxygen transfer to tissues in young and middle-aged healthy people*. Clin Hemorheol Micro. 2004; 30: 443–448.
- [9] Vetrugno M, Cantatore F, Arnese L, Delle Noci N, Sborgia C: *Red blood cell deformability, aggregability and cytosolic calcium concentration in normal tension glaucoma*. Clinical Hemorh Micro. 2004; 31: 295–302.
- [10] Maeda N: *Erythrocyte rheology in microcirculation*. Jap J Physiol. 1996; 46: 1–14.
- [11] Mohandas N, Shohet SB: *Red cell rheology*. Verlag-Springer, New York, 1978; 25–38.
- [12] Kochmański M, Zochowski RJ: *Lepkość krwi a mikrokążenie w mięśniu serca*. Kard Pol. 1990; 33: 64–70.
- [13] Sułek K: *Kontrola lepkości krwi – rzeczywistość i nadzieje*. Pol Arch Med Wew. 1984; 71: 207–214.
- [14] Słowińska L, Monkos K: *Kliniczne zastosowania laserowo-optycznego rotacyjnego analizatora krwinek czerwonych LORCA*, Annal Acad Med Siles. 2010; 64: (3–4) 42–47.
- [15] Zijlstra WG: *Syllectometry, a new method for studying rouleaux formation of red blood cells*, Acta Physiol Pharm. 1958; 7: 153–154.
- [16] Hardeman MR, Dobbe JGC, Ince C: *The laser – assisted optical rotational cell analyzer (LORCA) as red blood cell aggregometer*. Clin Hemorh Micro. 2001; 25: 1–11.
- [17] Hardeman MR, Goedhart PT, Dobbe JGC, Lettinga KP: *Laser-assisted optical rotational cell analyser (LORCA), A new instrument for measurement of various structural hemorheological parameters*. Clin Hemorheol. 1994; 14 (4): 606–618.

- [18] Baskurt OK, Hardeman M, Uyuklu M, Ulker P, Cengiz M, Nemeth N, et al.: *Parametrization of red blood cell elongation index – shear stress curves obtained by ektacytometry*. Scand J Clin Lab Invest 2009; 69: 777–788.
- [19] Baskurt OK, Meiselman HJ: *Analyzing shear stress-elongation index curves: comparison of two approaches to simplify data presentation*. Clin Hemorh Microirc. 2004; 31: 23–30.
- [20] Baskurt OK, Meiselman HJ: *Data reduction methods for ektacytometry in clinical hemorheology*. Clin Hemorheol Microcirc. 2013; 54 (1): 99–107.
- [21] Lubkowska A, Szygula Z: *Changes in blood pressure with compensatory heart rate decrease and in the level of aerobic capacity in response to repeated whole-body cryostimulation in normotensive, young and physically active men*. Int J Occup Med Env. 2010; 23: 367–375.
- [22] Kępińska M, Teległów A, Dąbrowski Z, Szygula Z: *Wpływ jednorazowej krioterapii ogólnoustrojowej na właściwości morfologiczne i reologiczne krwi u zdrowych mężczyzn*, VIII Konferencja Adeptów Fizjologii, Katowice 2013; 28–29.06.
- [23] Kępińska M, Szygula Z, Teległów A, Dąbrowski Z: *Impact of 24 Systemic Cryotherapy Treatments on the Rheological and Morphological Properties of Blood in Healthy Men*, 2nd International Congress on Sport Sciences Research and Technology Support, Rome, Italy 2014; 24–26.10.
- [24] Straburzyńska-Lupa A, Konarska A, Nowak A, Straburzyńska-Migaj E, Konarski J, Kijewski K et al. *Wpływ krioterapii ogólnoustrojowej na wybrane parametry biochemiczne krwi obwodowej zawodników hokeja na trawie*, Fizjo Pol. 2007; 7 (1):15–20.
- [25] Zagrobelny Z, Halawa B, Kuliczkowski K, Frydecka I, Gregorowicz H: *Wpływ ogólnoustrojowej krioterapii w komorze niskotemperaturowej oraz leczenia ruchem na subpopulację limfocytów we krwi obwodowej u chorych na chorobę zwyrodnieniową stawów i reumatoidalne zapalenia stawów*. Reumatol. 1996; 4: 763–771.
- [26] Banfi G, Melegati G, Barassi A, Dogliotti G, Melzi D'èril G, Dugue B et al.: *Effects of whole-body cryotherapy on serum mediator of inflammation and serum muscle enzymes in athletes*. J Therm Biol. 2009; 34: 55–59.
- [27] Banfi G, Krajewska M, Melegati G, Patacchini M: *Effects of whole body cryotherapy on haematological values in athletes*. BJSM. 2008; 42 (10): 858.
- [28] Klimek AT, Lubkowska A, Szygula Z, Chudecka M, Frączek B: *Influence of the ten sessions of the whole body cryostimulation on aerobic and anaerobic capacity*. Int J Occup Med Env. 2010, 23 (2): 181–189.
- [29] Lombardi G, Lanteri P, Porcelli S, Mauri C, Colombini A: *Hematological profile and material status in rugby players during whole body cryostimulation*. PLOS. 2013; 8(2) e55803. doi:10.1371/journal.pone.0055803
- [30] Lubkowska A, Suska M: *The increase in systolic and diastolic blood pressure after exposure to cryogenic temperatures in normotensive men as a contraindication for whole-body cryostimulation*. J Therm Biol. 2011; 36: 264–268.
- [31] Ziemann E, Olek RA, Kujach S, Grzywacz T, Antosiewicz J: *Fiveday whole-body cryostimulation, blood inflammatory markers, and performance in high-ranking professional tennis players*. JAT. 2012; 47 (6): 664–672.
- [32] Ziemann E, Olek R.A, Kujach S, Grzywacz T, Antosiewicz J: *Fiveday whole-body cryostimulation, blood inflammatory markers, and performance in high-ranking professional tennis players*, Journal of Athletic Training, 47(6), 664–672, 2012.
- [33] Blatteis CM: *Physiology and pathophysiology of temperature regulation*. World Scientific, 1998.
- [34] Teległów A, Dąbrowski Z, Marchewka A, Głodzik J, Rembiasz K, Krawczyk M et al.: *Effects of winter swimming and whole-body cryotherapy on the hematological and rheological properties of blood in regular winter swimmers and individuals exposed to whole-body cryotherapy*. Med Sport. 2014; 18 (2): 52–57.
- [35] Teległów A, Dąbrowski Z, Marchewka A, Tyka A, Krawczyk M, Głodzik J et al. *The influence of winter swimming on the rheological properties of blood*. Clin Hemorh Micro 2014; 57 (2): 119–27.

Word count: 4.427

Tables: 2

Figures: 1

References: 35

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