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IMPACT OF SYSTEMIC CRYOTHERAPY ON SELECTED ENZYMES, GLUTATHIONE AND SERUM TOTAL PROTEIN LEVELS 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
- G. Funds collection

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Abstract

Study aim. Determining the impact of systemic cryotherapy on the levels of AChE (acetylcholinesterases), G-6-PD (glucose-6-phosphate dehydrogenases), GSH (glutathione), plasma proteins and occult Hb (occult hemoglobin) in young healthy men.

Study group. The study group comprised of the 10 healthy men (untrained) aged 23–24 years (23.4 ± 0.52), who underwent cryotherapy treatments (treatment time 3 min, chamber temperature of -120°C , 10 treatment sessions – 5 times a week). The average body height was 179.2 ± 6.4 cm, weight 79.6 ± 8.8 kg, BMI 23.4 ± 2.6 kg/m². In order to analyze the levels of AChE, G-6-PD, GSH, occult hemoglobin and plasma proteins, venous blood was collected from the study participants three times. The first test (control) took place two months before cryotherapy, the second on the day of beginning treatment and the third test after a series of 10 treatments.

Methodology. Measurements were taken using the Helios Beta Z OP Spectro-Lab spectrophotometer, the VisionPro 4.10 Thermo Electron UV-Visible Spectroscopy program. To measure the concentration of AChE [U/gHb] and G-6-PD [U/gHb], the method proposed by Beutler (1986) was used. Plasma hemoglobin concentration [g/100 ml] was determined using the method by Chanazin (1989). The concentration of reduced glutathione GSH [mmol/gHb] was determined on the basis of the method developed by Ellman (1970). The Biuret method was used for the determination of total protein [g/l].

Results. Analyzing the average values of AChE [U/gHb], G-6-PD [mmol/gHb] and occult Hb. [g/dl] before and after the usage of systemic cryotherapy, no statistically significant differences were found. However, an increase in total protein concentration [g/l] and a decrease in GSH concentration [mmol/gHb] were found in the men who underwent cryotherapy in relation to the measurement taken two months before starting the treatment.

Conclusions. Systemic cryotherapy does not cause any side effects in young, healthy men. Regular usage of cryotherapy treatments may affect the levels of enzyme indicators – total protein (concentration increase) and glutathione (concentration reduction) in the blood.

Introduction

The impact of thermal factors on the human body has been studied for many years. Cold therapy is widely used in clinical practice and sports. The great

interest in cryotherapy (WBC – Whole Body Cryotherapy) causes the number extremely low temperature treatment centers to constantly increase [1, 2, 3]. The body's response to cold is based on hormonal changes [4, 5], in the cardiovascular system [6], nervous sys-

tem and muscles [7] and in the immune system [4, 5, 6, 8, 9]. Many studies have shown a relationship between whole-body exposure to extremely low temperatures and changes in the level of selected enzymes and hormones in body fluids [10]. Morphological and biochemical research carried out after application of cryotherapy is indicative of an increase in levels of hemoglobin, leucocytes and blood platelets compared to baseline values. There is also an increase in serum concentrations of epinephrine, norepinephrine, ACTH, cortisol, testosterone (in men), and a reduction of inflammatory parameters such as ESR, Waaler-Rose reaction, seromucoid [10, 11]. Also observed is the beneficial effect of cryotherapy on the cellular immunity of the body by increasing the number of Natural Killer cells [12, 13]. A series of biochemical antioxidant reactions are launched, thanks to which the adverse effect of free radicals are limited [14]. Despite the many possible health benefits posed by cryotherapy, little scientific information is available in literature, and the impact on laboratory results still requires much research. In our earlier study, we studied the effect of WBC on rheological-morphological indicators, in which we demonstrated the positive effects of these treatments [15]. We have decided to extend the determination by additional parameters.

The aim of the study was to determine the effects of systemic cryotherapy on the level of AChE (acetylcholinesterase), G-6-PD (glucose-6-phosphate dehydrogenases), GSH (glutathione), plasma proteins and occult Hb (occult hemoglobin) in young healthy men.

Study design

The study group consisted of 10 healthy males – Physiotherapy students of the University School of Physical Education in Krakow (no athletes; preliminary research – without calculation), aged 23–24 (23.4 ± 0.52), who underwent systemic cryotherapy treatments (10 treatments – 5 times a week). Their average body height was 178.9 ± 6.4 cm, weight 79.3 ± 6.8 kg and BMI 23.5 ± 2.4 kg/m². In order to analyze the AChE, G-6-PD, GSH, occult Hb and serum total protein levels, venous blood samples were drawn from the study participants three times. The first study was held two months before cryotherapy (control! – the control group consisted of the same persons who had taken part in the project), the second on the day of beginning treatments and the third test was conducted after a series of 10 treatments.

The parameters obtained in the cryo-chamber:

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

The time of a single treatment session for the group of males was 1.5 min (1st treatment), 3 min (2nd–10th treatment). 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 the 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.

Measurements were performed using the Helios Beta Z OP Spectro-Lab spectrophotometer, VisionPro 4.10 Thermo Electron UV-Visible Spectroscopy program. To measure the concentration of AChE [U/gHb] and G-6-PD [U/gHb], the method proposed by Beutler (1986) was used. Plasma hemoglobin concentration [g/100 ml] was determined using the method by Chanazin (1989). The concentration of reduced glutathione GSH [mmol/gHb] was determined on the basis of the method developed by Ellman (1970). The Biuret method was used for the determination of total protein [g/l].

Statistical analysis

The data is presented as mean and standard deviation ($\bar{x} \pm \text{SD}$) or median and first and third quartile depending on the normality of distribution which was verified using the Shapiro-Wilk test. The differences between the resulting measurements were analyzed by one way repeated measurements analysis of variance (ANOVA). Sphericity was assessed using Mauchly's test. In the case that the sphericity assumption was not met, the multidimensional test was used (Wilks' lambda). When the ANOVA parametric assumptions were not met, differences between the measurements were calculated using the Friedman test. Appropriate post-hoc tests were applied to evaluate the differences between particular measurements. The significance level was set at $p < 0.05$. Analyses were performed using Statistica 10 (StatSoft®, USA).

Results

Analyzing the average values of AChE [U/gHb], G-6-PD [mmol/gHb] and occult Hb. [g/dl] before and after the usage of systemic cryotherapy, no statistically significant differences were found. However, an increase in total protein concentration [g/l] and a decrease in GSH concentration [mmol/gHb] were found in the men who underwent cryotherapy in relation to the measurement taken two months before starting the treatment (Tab. 1).

Table 1. Mean values \pm standard deviation or median and (I-III quartile) of selected serum parameters in males before and after systemic cryotherapy

Cryotherapy	Measurement 1 (n = 10)	Measurement 2 (n = 10)	Measurement 3 (n = 10)	p	p1/2	p1/3	p2/3
Serum total protein [g/l]	65.71 \pm 4.66	68.52 \pm 8.45	72.71 \pm 6.62	0.013	0.381	0.038	0.198
AChE [U/gHb]	32.58 \pm 3.51	30.39 \pm 3.35	32.71 \pm 4.76	0.350			
G6PD [U/gHb]	0.41 \pm 0.09	0.43 \pm 0.14	0.47 \pm 0.10	0.626			
GSH [μ mol/gHb]	15.23 (13.17–16.89)	13.68 (13.45–14.53)	10.73 (5.65–12.00)	0.045	0.316	0.039	0.358
Occult Hb [g/dl]	0.000 (0.000–0.000)	0.005 (0.000–0.010)	0.000 (0.000–0.000)	0.156			

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

Conclusions

Systemic cryotherapy does not cause any side effects in young, healthy men. Regular usage of cryotherapy treatments may affect the levels of enzyme indicators – total protein (concentration increase) and glutathione (concentration reduction) in the blood.

Discussion

The research presented in this article is intended to show changes in the level of acetylcholinesterases, glucose-6-phosphate dehydrogenases, glutathione, occult hemoglobin and plasma proteins in young healthy men undergoing a series of 10 systemic cryotherapy treatments (-120°C). A review of literature shows lack of data on the effects of systemic cryotherapy on the levels of AChE, G-6-PD, GSH, occult Hb. and plasma proteins (especially in humans).

In our research, we have noted an increase in total protein concentration and a reduction in GSH concentration in the men undergoing cryotherapy treatments in relation to the measurements made two months before starting the treatment. However, there were no changes in the average values of AChE, G-6-PD or occult Hb.

In literature, there are few studies on the enzymatic properties of blood in cold water. Nonaka et al. (2012) showed an increase of G-6-PD in rats performing exercise, and then floating in cold water (4°C) [20]. In our study, we did not observe any changes in the activity of glucose-6-phosphate dehydrogenase.

Dede et al. (2002) showed that the reduced level of GSH in the blood of rats in a state of hypothermia is accompanied by reduced G-6-PD activity [21].

Puntel et al. (2013) studied rats with ischemia, compared to healthy subjects [22]. Both groups were subjected to cryotherapy (ice packs). In healthy subjects, no changes in GSH levels were observed.

Based on the research, decreased activity of glutathione in men using cryotherapy treatment was noted.

Even though Aslan and Meral (2007) used a “different kind of cold” and used the animal model (guinea-pigs with short-term hypothermia), they found the same relationship [23].

Pathological processes may be accompanied by both a reduction and increase in GSH concentration in particular tissues. Action aimed at increasing the level of GSH is beneficial in all these disease states, in which a decrease in GSH concentration occurs. However, a decrease in the level of GSH is recommended in tumor cells in order to increase their sensitivity to chemo- as well as radiotherapy, and also in organ transplantation to induce immunosuppression [24].

Research evaluating the effect of systemic cryotherapy in young men was conducted by Lubkowska et al. (2012) [25]. Its objective was to assess the effect of 20 systemic cryotherapy treatments on the antioxidant system. The author noted, inter alia, that only after 10 treatments, there was an increase in the total protein concentration. The same relationship was observed in the present study. The level of glutathione, however, increased after 10 treatments, and after 20 treatments, decreased compared to the level prior to WBC. In the conducted study, the opposite relationship was observed after 10 treatments.

The conducted research require extension to gain a better knowledge of the body's response in these conditions. Due to the small size of the study group, this research can be regarded as preliminary. The results of the studies carried out so far (very few) are difficult to interpret and compare because of differences in their research protocols. Lubkowska and Szyguła (2010) showed that the number of WBC sessions is of significance when considering the changes in morphological indicators [26].

In summary, these studies have reported that exposure to cold in the form of systemic cryotherapy can modulate the enzymatic parameters of the blood. It is believed that these results are important in determining the safety of WBC in the field of clinical trials.

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