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Enzyme activity: acetylcholinesterase and glucose-6-phosphate dehydrogenase in winter swimmers

Aktywność enzymów: acetylocholinesterazy i dehydrogenazy glukozo–6–fosforanowej u „Morsów”

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Key words
enzymes, acetylcholinesterase (AChE), glucose-6-phosphate dehydrogenase (G-6-PD), winter swimming

Abstract
Study aim: The aim of the study was to show changes in the activity of acetylcholinesterase (AChE) and glucose-6-phosphate dehydrogenase (G-6-PD) in winter swimmers between the end (April) and beginning of the consecutive winter swimming season (November).
Material and methodology: The study group consisted of 16 winter swimmers (non-training males) from the Krakow “Kaloryfer” [Radiators] Winter Swimming Club, regularly undergoing submersion in cold water at a temperature of 2-7.2ºC for a maximum of three minutes during the winter swimming season. The tests were carried out at the end and before the beginning of the following winter season using the method of spectrophotometry.
Results: Analysing the average values of enzymes after (April) and before the next (November) winter bathing season, there was a decrease in the activity of AChE [U/gHb] by 18.26% and G-6-PD [U/gHb] by 22.11% in men undergoing winter baths.
Conclusions: Regular use of winter bath treatments results in increased enzyme activity: AChE and G-6-PD; and while break in winter swimming reduces the activity of these enzymes.

Słowa kluczowe
enzymy, acetylocholinesteraza (AChE), dehydrogenaza glukozo-6-fosforanowa (G-6-PD), morsowanie

Streszczenie
Cel badań: Celem pracy było wykazanie zmian aktywności acetylocholinesterazy (AChE) i dehydrogenazy glukozo-6-fosforanowej (G-6-PD) u „Morsów” między końcem (kwiecień) a początkiem następnego sezonu morsowego (listopad).
Materiał i metodyka: Grupę badaną stanowiło 16 „Morsów” (nietrenujący mężczyźni) z Krakowskiego Klubu Morsów „Kaloryfer”, regularnie poddający się zanurzeniu w zimnej wodzie o temperaturze 2-7,2ºC na czas nie dłuższy niż 3 minuty podczas sezonu morsowego. Badania przeprowadzano po zakończeniu oraz przed rozpoczęciem kolejnego sezonu zimowego przy użyciu metody spektrofotometrycznej.
Wyniki: Analizując średnie wartości enzymów po (kwiecień) i przed kolejnym (listopad) sezonem zimowych kąpieli zanotowano zmniejszenie aktywności AChE [U/gHb] o 18,26% i G-6-PD [U/gHb] o 22,11% u mężczyzn korzystających z zimowych kąpieli.
Wnioski: Regularne korzystanie z zabiegów zimowych kąpieli wpływa na zwiększenie aktywności enzymów: AChE i G-6-PD, a przerwa w morsowaniu powoduje obniżenie aktywności tych enzymów.

The individual division of this paper was as follows: a – research work project; B – data collection; C – statistical analysis; D – data interpretation; E – manuscript compilation; F – publication search

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INTRODUCTION

Subjecting oneself to cold baths during the winter season is now an increasingly well-known physical activity, which thanks to the many benefits that it brings to man, can be referred to as an unconventional way to maintain health. Exposure to cold is a strong environmental stressor leading to many significant physiological reactions. Whole-body, systemic, cold baths contribute to the occurrence of a kind of thermal shock, thus stimulating the vascular system. Cooling the body in water occurs two to three times faster than in the air. We expect less susceptibility to cold in the case of people taking part in regular ice-water bathing. It has been proven that cold baths prevent diseases, infections and toughen the body. In winter swimmers, an increased immune system response can be observed. Winter swimming can also contribute to a reduction in pain for more than 24 hours. A single bath in cold water causes an increase in sympathetic nervous system activity, resulting in a four-fold increase in noradrenaline plasma concentration, while the activity of the renin-angiotensin system drops by half. The concentration of other catecholamines also increases, which positively affects the increase in cellular metabolism. However, after adapting the organism to cold water, diastolic blood pressure is reduced and peripheral vasospasm increases. The negative reaction to immersion in cold water may be apnea and hyperventilation. In the circulatory system, we may observe an increase in haemoglobin content by 10-20%, an increase in haematocrit, platelet count and glucose concentration, while a reduction in the number of white blood cells occurs. Thanks to the body's exposure to cold water, erythrocytes become more flexible, which is associated with better oxygenation of the body. Regular use of cold baths exposes us to the occurrence of oxidative stress to a lesser extent through changes in the content of GSH (reduced glutathione) and GSSG (oxidized glutathione) as well as uric acid[1-7].

The aim of the study was to show changes in the activity of acetylcholinesterase (AChE) and glucose-6-phosphate dehydrogenase (G-6-PD) in winter swimmers between the end (April) and beginning of the consecutive winter swimming season (November). To achieve the above-posed goal, the following research question was established: did the break in winter swimming cause changes in the AChE and G-6-PD indices among the winter swimmers?

MATERIAL AND METHODOLOGY

The study group consisted of 16 winter swimmers (non-training men) from the Kraków "Kaloryfer" [Radiators] Winter Swimming Club, who regularly underwent cold water baths at a temperature between 2°C and 7.2°C for a maximum of 3 minutes during the winter swimming season. The tests were carried out after completion (April) and before the beginning of the following winter season (November). Blood was collected from the subjects in a fasting state during the morning hours, in the amount of 3 ml from the ulnar vein and was put into test tubes with EDTAK.2. The blood was collected by a qualified nurse, under the supervision of a physician and in accordance with the applicable standards at the Department of Motor Pathology of the University of Physical Education in Kraków. The tests were approved by the Bioethical Commission at the District Medical Chamber in Krakow.

Measurements were conducted using the Helios Beta Z OP Spectro-Lab Spectrophotometer, VisionPro 4.10 Thermo Elektron UV-Visible Spectroscopy. For the measurement of AChE [U/gHb] and G-6-PD [U/gHb] concentrations (in erythrocytes - RBC), the Beutler method (1986)was used[8].

The G-6-PD enzyme catalyses the oxidative reaction of glucose-6-phosphate (G-6-P) to 6-*phosphoglucono-lactone*. During this reaction, NADP is reduced via the enzyme within 10 minutes. Then, the absorption increment was measured at a wavelength of 340 nm. The following reagents were used: Tris-HCl (1M) EDTA (5mM, pH=8.0) - 100 μl; $MgCl_2$ (0.1 M) -100 μl; NADP (2 nM) – 100 μl; hemolysate – 20 μl; distilled water – 580 μl; and successively, incubation: 37°C, 10 min and G-6-P (6nM) – 100 μl.

The AChE activity assay method uses the acetylcholine hydrolysis reaction to the thiocholine compound catalysed by AChE. The increase is measured by the reaction of thiocholine with *5,5'Dithiobis(2-nitrobenzoic acid*) (DTNB). The product of this reaction is 5-thio-2-nitrobenzoic acid, characterized by its yellow colour, the product concentration is measured spectrophotometrically at a wavelength of 412 nm. The test was prepared by adding the following in the order: Tris-HCL (1M) EDTA (5 mM, pH = 8.0) – 100 μl; DTNB (0.5 mM in 1% sodium citrate) – 50 μl; hemolysate diluted in distilled water (1:10) – 10 μl; distilled water – 790 μl; and successively, incubation: 37°C, 10 min and acetylthiocholine iodide (10 mM) – 50 μl.

Statistical analysis

The test results were analysed using the STATISTICA 10 programme, StatSoft (USA). Compliance of the obtained data with normal distribution was verified using the Shapiro-Wilk test. Quantitative variables are represented by mean and standard deviation ($\bar{x} \pm SD$). In order to analyse parameter changes in the group of subjects, the Student's *t*-test for dependent variables was used. The significance level of $\alpha = 0.05$ was assumed.

RESULTS

Analysing the average values of enzymes after (April) and before the following (November) winter swimming season, there was a decrease in the activity of AChE [U/gHb] by 18.26% and G-6-PD [U/gHb] by 22.11% in males taking winter baths (Table 1, Figures 1 and 2).

Table 1

Mean values ± standard deviation (*SD*) of enzymatic activity at the end and beginning of the consecutive winter swimming season in men undergoing winter baths

	End of the season	Beginning of the season	*p*	difference %
AChE [U/g Hb]	17.36 ± 4.15	14.19 ± 2.98	**0.0027**	**18.26**
G-6-PD [U/g Hb]	1.90 ± 0.70	1.48 ± 0.36	**0.0211**	**22.11**

N = 16 subjects

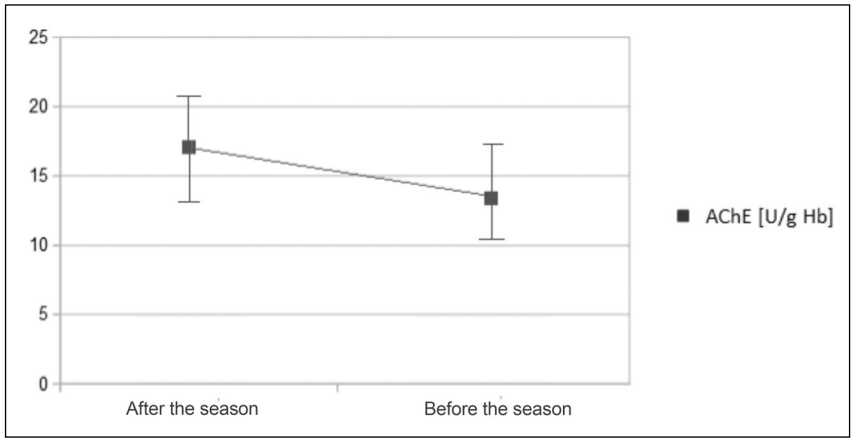

Figure 1

AChE [U/g Hb] at the end and beginning of the following winter swimming season in men undergoing winter baths (N = 16 subjects)

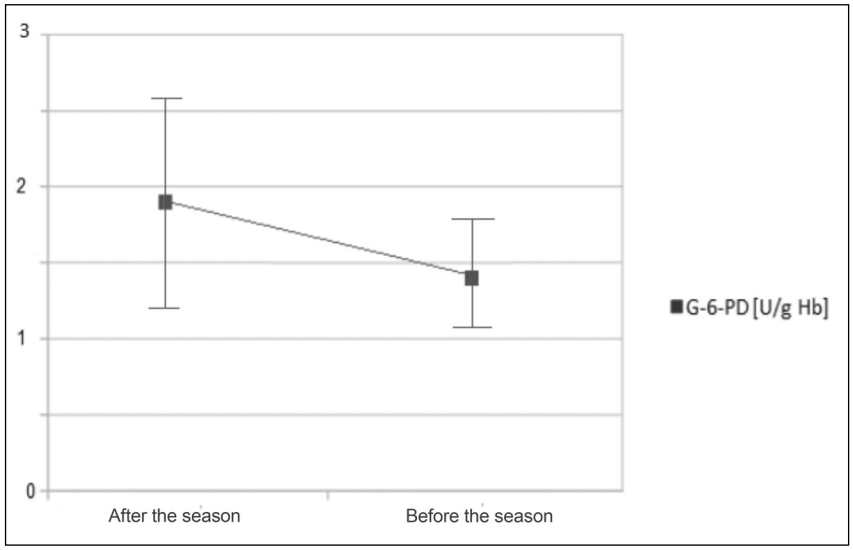

Figure 2

G-6-PD [U/g Hb] at the end and beginning of the following winter swimming season in men undergoing winter baths (N = 16 subjects)

DISCUSSION

The aim of this study was to show changes in AChE and G-6-PD activity after the winter period in which winter swimming was performed. During exposure to extremely low temperatures, mechanisms compensating for heat loss in the body are activated[9]. In an aquatic environment, heat loss occurs faster than in cold air, and its loss is extremely rapid and stressful for the body[10,11]. A review of literature indicates lack of data on the effects of winter swimming and mid-season breaks on enzymatic indices in men undergoing winter baths.

The biological significance of AChE, found in the membrane of red blood cells (RBC), is not fully explained. However, it is known that the erythrocytic enzyme has many similarities to the enzyme found in brain tissue[12]. The AChE in the synaptic clefts metabolizes the acetylholine neurotransmitter to choline and acetyl residue. In the parasympathetic nervous system, this enzyme plays a major role in the transmission and formation of nerve impulses. Closely related to the erythrocytic membrane, AChE is one of the most important proteins able to ensure the integrity of the red cell membrane. The outer-membrane location of the enzyme makes it susceptible to the presence of oxidants in the body that affect the membrane structure of the blood cells[17]. The effectiveness of AChE membrane activity is largely due to the parameters of the blood cells and cell membrane. This activity is regulated by the hydrophobic environment of the erythrocyte membrane and depends on fluidity of the membrane as well as the charge on the erythrocyte surface[13]. AChE located in the RBC membrane is extremely susceptible to so-called oxidative stress. Too many unneutralized oxidants in the blood may reduce AChE activity in many cases of its action, i.e., influence on the erythrocyte membrane. Following the interaction of free radicals, inhibition of AChE enzyme activity may occur[14]. Both changes in the rheological properties of the cell membrane and antioxidant may contribute to the reduction of acetyl farinesterase activity in RBC[15]. In addition, due to the fact that AChE activity decreases with age, this enzyme is considered an enzymatic marker, and its activity may be an indicator of changes in the erythrocyte mem-

brane[13]. The decrease in AChE activity is significantly correlated with increased lipid peroxidation during the aging of red blood cells[16].

G-6-PD is an enzyme that catalyses the conversion of glucose-6-phosphate to 6-phosphogluconate in the presence of an oxidized form of nicotinamide adenine dinucleotide phosphate (NAPD), being the first enzyme of the pentose pathway. From the point of view of antioxidant enzyme activity, G-6-PD is an enzyme that is significantly associated with reduced glutathione. The overriding function of G-6-PD is to reduce the form of nicotinamide adenine dinucleotide phosphate (NADPH) and ribose-5-phosphate, which play a significant role in the biosynthesis of nucleic acids and membrane lipids[17]. These processes play a key role in the properties and functioning of the erythrocyte membrane, protecting the so-called thiol groups of membrane proteins from oxidation[18]. NADPH is a very important element in the body's antioxidant and red blood cell defense[10]. It ensures the renewal of reduced glutathione resources, which prevents haemoglobin oxidation while guaranteeing integrity of erythrocyte cell membrane sulfhydryl groups taking part in the detoxification of hydrogen peroxide and oxygen radicals in RBC. In case of G-6-PD deficiency, erythrocytes are extremely vulnerable and susceptible to oxidation and are rapidly haemolysed in the spleen[15,18,19].

The research and results presented in this work demonstrate the impact of an environment extremely unfriendly to the body (leading to hypothermia) on the activity of two extremely important enzymes: AChE and G-6-PD. The human body being warm-blooded, maintains a constant internal temperature regardless of the conditions prevailing outside of it so that homeostasis is maintained also thanks to the control of the autonomic system. The body regulates the temperature of internal and external organs by means of the thermoregulation centre[3,20].

In adaptation to an external environment of extremely low temperatures, the main role is played by the acceleration of body metabolism, the consequences of which may be increased acetylcholinesterase and G-6-PD activity, enzymes strongly related to the metabolism of the body. Accelerated metabolism and exposure to extremely low temperatures have impact on increased oxygen consumption, and consequently, increased production of so-called oxygen free radicals in which G-6-PD is involved in the neutralization of the antioxidation process, which may explain the increased activity of this enzyme in winter swimmers[19, 20].

Applying winter baths affects the activity of G-6-PD, and thus, the pentose phosphate pathway in which G-6-PD plays a key role. It also takes part, among others, in the synthesis of steroids in adrenal, testicular and ovarian cells as well as in the synthesis of fatty acids in liver cells, adipose tissue and lactate glands[14,21]. There are as many as 400 genetic variants of G-6-PD deficiency translating into diseases, such as premature erythrocyte breakdown. Winter swimming, as a natural method increasing the activity of the G-6-PD enzyme, may be one of the natural methods to compensate for deficiencies in this enzyme, alleviating the symptoms of its deficiency and, at the same time, being an alternative to other treatments for people with G-6-PD lacks. However, no detailed research or publications on this subject are available. What is certain, however, is that winter swimming increased the activity of glucose-6-phosphate dehydrogenase, which may indicate that winter baths indirectly affect the antioxidant capacity of the body by increasing G-6-PD activity[21,22,23].

On the basis of the research presented in this paper, statistically significant changes in AChE and G-6-PD activity were found, which confirms that the body's adaptation to a new environment causes, among others, increased oxidative stress, and forced elevated metabolism increases the activity of these enzymes in the body in order to maintain homeostasis of the body. These results are believed to be important in determining the "suitability" of winter bathing in the field of clinical trials. However, research needs to be expanded to better understand the body's response under these conditions.

CONCLUSIONS

Regular use of winter bath treatments results in increased activity of the following enzymes: AChE and G-6-PD, while a break in the winter swimming reduces the activity of these enzymes.

Conflict of interest: None declared

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