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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

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Abstract

Introduction: Exposure to cold is one of the strongest physiological and psychological environmental stressors and induces an array of significant functional responses.

Objective: To analyze the changes in morphological and rheological parameters of blood in regular winter swimmers and individuals exposed to whole-body cryotherapy.

Methods: The study covered a period of two months (February and March) and included two groups of healthy males: 1) 10 winter swimmers who immersed in cold waters (3 min at 2°C to 7.2°C) once a week, and 2) 10 healthy volunteers who were exposed to cryotherapy (3 min at -110°C) on a weekly basis. Venous blood for morphological, biochemical (glucose, fibrinogen) and rheological analysis (aggregation index, the amplitude and total extent of aggregation, the half time of the aggregation) was sampled prior to the experiment, as well as after one and two months of regular exposure to cold.

Results: After two months of winter swimming, significant reduction of plasma fibrinogen was documented as compared to the baseline level. In contrast to winter swimmers, after two months of cryotherapy plasma concentration of fibrinogen was significantly higher than prior to the experiment. Moreover, significant increase in platelet count and the reduction in glucose concentration were documented after two months as compared the first month of cryotherapy.

Conclusions: This study confirmed that exposure to cold can modulate morphological and biochemical parameters of blood. Despite the lack of unfavorable changes in hemorheological indices of both studied groups, an increase in fibrinogen concentration documented in cryotherapy group points to potential risk associated with this form of cold exposure.

Keywords: *blood morphology; cryotherapy; erythrocyte; rheology; winter swimming*

Introduction

Exposure to cold belongs to the strongest physiological and psychological environmental stressors and induces an array of significant functional responses. Staying in cold aquatic environment is particularly demanding from the viewpoint of thermoregulatory processes. The rate of body cooling in water is 2- to 3-fold higher than in the air, and the relevant thermal loss is approximately 250-fold greater. The circulatory system, as well as sweat glands, skeletal muscles, adipose tissue, and liver include the most important effector structures of thermoregulatory system in humans. It is generally believed that it is the head, which represents the main site of thermal loss [1]. However, according to Hayward and Keatinge [2], also the neck, sternal region, lateral surfaces of the chest, and inguinal area are characterized by relatively high degree of thermal loss.

Whole-body cryotherapy (WBC) refers to the therapeutic application of temperatures below -110°C. Although a single WBC session lasts no longer than 2-3 minutes, it is reflected by significant, region-specific changes in skin temperature; in contrast, the changes in the core body temperature of individual staying at cryotherapeutic chamber do not exceed the thermoregulatory range. Cryotherapy is reflected by the active

hyperemia of tissues exposed to ultralow temperatures; this enhances their metabolism, stimulates the rate of byproduct removal, and reduces muscular tone. These changes result in documented anti-inflammatory and anti-edematous properties of WBC. Equally important are positive psychological changes induced by WBC: antidepressant effect, improvement of mood, and reduction of fatigue [3].

Cold water swimming (CWS) pertains to recreational exposure to low temperatures in aquatic environment. Regular CWS has been proven to be associated with several benefits to both somatic and mental health [4]. Individuals who participate in cold water immersion and swimming are characterized by higher resistance to respiratory tract infections. Lower prevalence of various disorders in subjects accustomed to swimming in cold water is associated with higher plasma concentrations of IgA [5,6].

A key phenomenon responsible for adaptation of winter swimmers to cold water immersion is the decreased dermal perfusion leading to improved thermo-isolating function of the skin [7,8]. The lower perfusion, in turn, results from a decreased cardiac output and cardiac rate, as well as the constriction of dermal blood vessels [9,10].

Sparse number of published reports has addressed the differences between physiological responses of individuals practicing CWS and those exposed to WBC. Leppaluoto et al. [11] analyzed a group of healthy subjects exposed to CWS (20 s at 0-2°C) and another group exposed to WBC (2 min at -110°C). A similar degree of sustained cold-induced stimulation of norepinephrine was documented in both groups. The authors concluded that this increase in norepinephrine might have a role in cold-induced pain alleviation [11]. Dugue et al. [12] studied the effects of severe cold stress on total peroxyl radical trapping antioxidant capacity of plasma (TRAP) in two groups of healthy women regularly exposed to cold during WBC or CWS. During the first four weeks of the experiment, the mean TRAP value increased significantly shortly after cold exposure and returned to its baseline level approximately 30 minutes thereafter. However, the cold-induced changes of TRAP were no longer observed after four weeks of CWS or WBC. Consequently, it was concluded that neither WBC nor CWS are harmful to plasma antioxidative capacity if practiced regularly [12].

Little is known about the cold-induced hemorheological changes. It was revealed that adaptation of circulatory system to CWS includes changes in the rheological properties of erythrocytes, particularly deformability under shear force. These adaptations are essential in preventing flow resistance and aggregation in the microcirculation, where erythrocytes correspond to nearly half of the volume of blood [13]. Although vitally important, the degree of abovementioned adaptive changes has not been studied comprehensively in winter swimmers. Similarly, to the best of our knowledge, there is no published research addressing the problem in question in individuals subjected to WBC.

Consequently, the aim of this study was to analyze the changes in morphological and rheological parameters of blood in regular winter swimmers and WBC-exposed individuals.

Methods

Participants

The study covered a period of two months (February and March) and included two groups of healthy males: 1) winter swimmers (n=10, all between 30 and 35 years of age) from Cracow Society of Winter Swimmers "Kaloryfer", who immersed in cold waters (3 min at 2°C to 7.2°C) of the Zakrzówek Lake once a week, and 2) 30- to 35-year-old healthy volunteers (n = 10), who were exposed to cryotherapy (3 min at -110°C) on a weekly basis.

Both the study protocol and all the procedures were approved by the Local Ethics Committee in Cracow.

Laboratory procedures

The samples of venous blood were obtained after an overnight fast, prior to the experiment, as well as after one and two months of regular exposure to cold. Overall, 60 samples were examined.

Blood collected in 10 µl tubes was used to determine basic hematological parameters. Testing was performed using analyzer for blood (ABX Diagnostics, France). The following parameters were determined: 1) red blood cell count (RBC, 10^{12}/L), 2) hemoglobin (HGB, g/L), 3) hematocrit (Ht, L/L), 4) mean corpuscular hemoglobin (MCH, fmol), 5) mean corpuscular volume (MCV, fL), 6) mean cell hemoglobin concentration (MCHC, mmol/L), 7) white blood cell count (WBC, 10^9/L), and 8) platelet number (PLT, 10^9/L).

The aggregation of red blood cells was determined using a Laser-assisted Optical Rotational Cell Analyzer (LORCA, RR Mechatronics, Holland) according to Hardeman et al. [14,15]. The following aggregation parameters were estimated: 1) aggregation index (AI, %), 2) the amplitude and total extent of aggregation (AMP, arbitrary units), and 3) the half time (T½, s), which describes the kinetics of the aggregation process and is proportional to the time of re-aggregation of disintegrated red cell complexes. Measurements of aggregation parameters were carried out on native hematocrit. The temperature in LORCA was adjusted to 37°C, while all other procedures and measurements were carried out at room temperature (22 ± 1°C). Aggregation measurements

obtained from LORCA aggregometer were based on the detection of laser backscattering from the sheared (disaggregated) and unsheared (aggregated) blood using a computer assisted system. Each 2 ml blood sample was transferred into a glass vessel and oxygenated for 10 to 15 minutes prior to obtaining measurements. A 1 ml sample of blood was injected into the gap between the outer cylinder "cup" and inner cylinder "bob" of LORCA. During the measurement, the cup was driven by a computer-controlled stepper motor. The blood sample was sheared at 400 s^{-1}, with shear rate decreasing rapidly to zero. The backscattering data was evaluated by the computer and the AI was calculated from the syllectrogram (light scatter vs. time curve during a 120 s period). This method relies on the fact that there is less light backscattered from aggregating red cells.

The viscosity of blood plasma was determined in a viscosimeter (Myrenne, Germany) with results displayed as mPa. Blood glucose concentrations were determined with the stripe method on Optium Xido device (Abbott Laboratories, Poland). The results were displayed as mmol/l. Concentrations of fibrinogen were determined with Chrom 7 coagulometer (Slamed Ing GmbH, Germany), with results displayed as g/l.

Statistical analysis

Continues variables were presented as medians and interquartile ranges. The normality of distribution was tested using the Shapiro-Wilk test. Consecutive values of analyzed parameters within the groups were compared with Friedman ANOVA and Wilcoxon's test. Mann-Whitney test was used for intergroup comparisons. Calculations were performed using Statistica 7 (StatSoft®, Poland) software, and statistical significance was defined as $P \leq 0.05$.

Results

Winter swimming group

After two months of winter swimming, significant reduction in plasma fibrinogen was documented as compared to the baseline level. In contrast, no significant CWS-related changes in blood morphology as well as in the median concentration of glucose, blood plasma viscosity, and aggregation indices were documented throughout the period of this study (Table 1).

Cryotherapy group

In contrast to winter swimmers, after two months of cryotherapy plasma concentration of fibrinogen was significantly higher than prior to the experiment. Moreover, a significant increase in platelet count and a reduction in glucose concentration were documented after two months as compared the first month of cryotherapy (Table 2).

Intergroup differences

At baseline, winter swimmers were characterized by significantly higher platelet count as compared to the cryotherapy group ($P = 0.046$). Additionally, this group predominated over the cryotherapy group in terms of fibrinogen concentration at baseline ($P = 0.004$) and after one month of the study ($P = 0.036$), as well as glucose concentration at the end of the experiment ($P < 0.000$).

Table 1. *Median (interquartile ranges) of basic hematological parameters, glucose and fibrinogen concentrations, and hemorheological indices determined in 10 males prior to and after one and two months of regular winter swimming*

Parameter	Month 0	Month 1	Month 2	*P*-value
RBC (10^{12}/L)	5.11 (4.5-6.06)	5.21 (4.31-5.84)	4.89 (4.49-5.62)	ns
Hb (g/L)	14.85 (13.1-16.3)	15.6 (13.7-19.0)	15.3 (13.8-15.7)	ns
Ht (L/L)	45.8 (42.6-50.5)	45.5 (42.1-50.0)	45.9 (42.7-50.6)	ns
MCHC (mmol/L)	32.05 (31.4-32.9)	31.8 (31.5-33.9)	31.6 (30.9-32.2)	ns
MCH (fmol)	29.00 (26.3-31.5)	28.9 (26.6-33.2)	29.4(28.0-31.1)	ns
MCV (fL)	90.5 (83.0-98.0)	91.0 (84.0-98.0)	93.0 (89.0-98.0)	ns
WBC (10^9/L)	5.3 (3.7-8.7)	5.1 (4.1-6.3)	4.4 (3.9-8.9)	ns
PLT (10^9/L)	233.0 (199.0-286.0)	211.0 (192.0-265.0)	204.0 (171.0-250.0)	ns
Glucose (mmol/l)	106.0 (86.0-118.0)	102.0 (89.0-113.0)	105.0 (99.0-117.0)	ns
Fibrinogen (g/l)	4.59 (3.59-5.27)	4.31 (3.85-5.58)	3.94 (2.91-4.45)*	0.027
BPV (mPa)	1.24 (1.15-1.45)	1.19 (1.11-1.64)	1.16 (1.09-1.28)	ns
AI (%)	61.11 (45.71-66.32)	64.55 (52.02-70.08)	62.27 (53.5-68.74)	ns
T½ (s)	2.32 (1.81-4.79)	1.95 (1.43-3.5)	2.22 (1.63-3.33)	ns
AMP (au)	21.35 (17.29-25.09)	19.09 (17.05-22.57)	20.27 (17.44-22.27)	ns

*significantly lower as compared to Month 0; ns – not significant

Table 2. *Median (interquartile ranges) of basic hematological parameters, glucose and fibrinogen concentrations, and hemorheological indices determined in 10 males prior to and after one and two months of regular exposure to whole-body cryotherapy*

Parameter	Month 0	Month 1	Month 2	*P*-value
RBC (10^{12}/L)	4.95 (4.35-5.4)	5.15 (4.7-5.76)	5.03 (4.59-5.55)	ns
Hb (g/L)	14.85 (13.1-16.3)	15.6 (13.7-19.0)	15,3 (13.8-15.7)	ns
Ht (L/L)	46.4 (40.2-49.7)	48.7 (42.7-50.8)	45.6 (42.5-48.4)	ns
MCHC (mmol/L)	32.75 (33.3-33.3)	32.2 (31.4-37.4)	32.9(31.9-36.1)	ns
MCH (fmol)	29.95 (27.7-32.1)	29.95 (28.0-33.0)	30,2(28.1-34.0)	ns
MCV (fL)	92.5 (88.0-98.0)	91.0 (88.0-96.0)	91.0 (87.0-94.0)	ns
WBC (10^9/L)	5.3 (3.7-8.7)	5.1 (4.1-6.3)	5,17(4.4-3.9)	ns
PLT (10^9/L)	178.5 (144.0-274.0)	200.5 (150.0-265.0)	215.0(159.0-281.0)*	0.050
Glucose (mmol/l)	100.0 (87.0-109.0)	104,0 (72.0-129.0)	72.0 (67.0-92.0)*	<0.001
Fibrinogen (g/l)	3.74 (3.37-4.28)	3.78 (3.21-4.37)	4.02 (3.23-5.45)**	0.014
BPV (mPa)	1.18 (1.03-1.58)	1.17 (1.01-1.32)	1.56 (1.02-1.8)	ns
AI (%)	54.52 (43.52-58.02)	54.79 (47.19-92.39)	55,48 (42.46-64.81)	ns
T½ (s)	3.22 (2.72-5.12)	3.2 (0.03-4.42)	3,04 (1.87-5.32)	ns
AMP (au)	19.89 (13.52-23.24)	20.07 (12.38-23.55)	17.14 (14.26-21.68)	ns

significantly different as compared to: *Month 1, **Month 0

Discussion

It is believed that both winter swimming and cryotherapy can improve immunity. Regular exposure to low temperatures is reflected by an increased tolerance to cold. Exposure to low temperatures results in the activation of thermoregulatory mechanisms; increased metabolic rate, as well as reduced perfusion of dermal vessels, and decreased heart rate are observed immediately after immersing in ice-cold water or entering cryogenic chamber. These changes are followed by prolonged reflex increase in heart rate, and dilatation of blood vessels, resulting in hyperemia and enhanced supply of oxygen, nutrients, and anti-inflammatory factors. The resultant persistent hyperemia exerts analgesic and anti-inflammatory effects. Additionally, it promotes muscular relaxation and injury healing, improves joint mobility, and resolves edema [16-19].

Determination of deformability and aggregation characteristics of erythrocytes enables the evaluation of their rheological behaviors. Rheological properties, such as deformation by shear force leading to reduced flow resistance, and aggregation resulting in the increase of flow resistance, are important determinants of microcirculation, where erythrocytes occupy nearly half of the volume [13]. Deformability, an important hemorheological parameter enabling the exchange of metabolic products with tissue environment, is determined by the composition of erythrocyte membrane and hemoglobin contents [20].

Squeezing through minute blood vessels with diameters smaller than 3-5 μm requires the deformation of human erythrocytes (7-8 μm in diameter). In this study, we found no significant changes in erythrocyte aggregation indices of winter swimmers and individuals exposed to cryotherapy. This suggests that either forms of exposure to low temperatures do not negatively affect blood rheology. Our previous studies documented the effects of a single bout of swimming to exhaustion on erythrocyte deformability and aggregation, as well as on fatty acid composition of erythrocyte membranes, in untrained older rats [21]. When compared to the control group, exercise performed in water at 4°C led to an increase in the elongation index (EI, from 0.30 Pa to 4.24 Pa) with no concurrent changes in erythrocyte aggregation, blood plasma viscosity, and fatty acid composition of erythrocyte membrane. In rats swimming in water at 25°C, we observed an increase in EI at shear stress from 0.30 Pa to 2.19 Pa along with a decrease in the half-time of total aggregation when compared to the control group [22]. Yalcin et al. [23] studied the effects of swimming on the rheological properties of blood in untrained rats. In their study, swimming resulted in a decrease in erythrocyte aggregation, starting 30 minutes after the exercise [23]. In contrast, Kayatekin et al. [24] revealed an increase in erythrocyte aggregation immediately after acute swimming.

In the present study, regular short-term exposure to cold during winter swimming and whole-body cryotherapy did not induced any unfavorable changes in hematological parameters of healthy participants. Two months of winter swimming were not reflected by any significant changes in blood morphology. In contrast, such changes were previously documented by Lombardi et al. [25], who revealed that winter swimmers are characterized by significantly higher number of erythrocytes, platelets, and leukocyte subpopulations:

neutrophils, lymphocytes, and monocytes. According to these authors, the elevated blood cell counts reflect direct induction of hematopoiesis at the bone marrow level under the pull of the sympathetic activity via the activation of a series of hormonal mediators, regulating the energy metabolism and the cold adaptation, and among them, leptin [25].

After two months of winter swimming, the concentration of fibrinogen in our participants was lower than prior to the study. In contrast, two months of regular cryotherapy were reflected by significant increase in fibrinogen concentration. Consequently, cryotherapy was proved a strong stimulator of fibrinogen synthesis. It is postulated that, due to persistent stimulation of macrophages, cryotherapy stimulates the synthesis of acute-phase mediators, including interleukin-6. In turn, this latter cytokine is known to enhance the synthesis of fibrinogen through the activation of transcription factors for fibrinogen beta-chain gene.

Aside from elevated plasma viscosity, higher concentrations of fibrinogen can modulate rheological properties of blood. In the presence of high-molecular-mass proteins (e.g. fibrinogen), erythrocytes lose their biconcave shape, which promotes stack (rouleaux) formation. Additionally, higher levels of fibrinogen favor formation of erythrocyte aggregates since this protein acts as an intercellular bridge [26, 27]. Consequently, it could be expected that cryotherapy-induced elevated concentrations of fibrinogen will be reflected by unfavorable changes in hemorheological parameters. However, such negative changes were not observed in our cryotherapy group.

Other significant changes documented in the group of individuals regularly exposed to cryogenic temperatures included an increase in blood platelet count as well as a decrease in glucose concentration. Plausibly, this latter phenomenon resulted from the cold-induced enhancement in the consumption of this sugar.

In conclusion, this study confirmed that exposure to cold can modulate morphological and biochemical parameters of blood. Despite the lack of unfavorable changes in hemorheological indices of both studied groups, an increase in fibrinogen concentration documented in cryotherapy group points to a potential risk associated with this form of cold exposure.

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Declaration of interest

The authors report no conflicts of interest.

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