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REGULAR COLD WATER SWIMMING DURING WINTER TIME AFFECTS RESTING HEMATOLOGICAL PARAMETERS AND SERUM ERYTHROPOIETIN

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Recreational winter swimming in cold sea water evokes body responses to regularly repeated cold water immersion. However, the understanding of adaptive changes is still limited and data regarding very short-term exposure to severe cold stress are scarce. The purpose of the study was to examine the effects of regular active cold water exposure on resting blood elements and erythropoietin in male and female cold water swimmers (CWSs). Thirty four healthy subjects (18 men and 16 women) aged 50.0 ± 12.2 years were swimming in cold sea water during winter season at least twice a week. The average water temperature was 9.5°C in October, 1.0°C in January and 4.4°C at the end of April. Fasting blood samples were taken within the first weeks of October, January and April. Serum erythropoietin (EPO), complete blood count (CBC) including evaluation of: red blood cells (RBC count, hemoglobin, hematocrit and RBC indices), white blood cells (WBC count with WBC differential), platelets (PLT count), serum folate and serum immunoglobulins (IgG, IgA, IgM) were determined. Between October and April an increase was observed in the following parameters: RBC (from 4.8×10^{12}/L to 5.2×10^{12}/L, $P < 0.001$), hemoglobin (from 8.6 mmol/L to 9.4 mmol/L, $P < 0.001$), MCH (from 1.8 fmol to 1.9 fmol, $P = 0.003$), MCHC (from 19.9 mmol/L to 20.6 mmol/L, $P < 0.001$), EPO (from 6.3 IU/L to 8.1 IU/L, $P = 0.001$). At the same time decreased concentrations of PLT (from 249.9×10^9/L to 221.6×10^9/L, $P = 0.005$), folate (from 10.5 ng/mL to 7.4 ng/mL, $P < 0.001$) and immunoglobulins (IgG: from 11.8 g/L to 10.9 g/L, $P < 0.001$; IgA: from 2.5 g/L to 2.2 g/L, $P < 0.001$; IgM: from 0.9 g/L to 0.8 g/L, $P < 0.001$). Statistically significant changes in EPO and PLT values were noted only in female CWSs. We conclude that regular cold water swimming induces adaptive changes in the resting blood elements and EPO concentrations which are more evident in female organism.

Key words: *cold water swimming, physical activity, erythropoietin, complete blood count, hemoglobin, white blood cells, platelets, immunoglobulins*

INTRODUCTION

Winter swimming is a form of physical activity associated with short lasting repeated bathing in a cold water reservoirs during the winter season. In recent years, this type of recreation has risen in popularity in certain countries, including Poland (1). Exposure to cold water can lead to different physiological responses, depending on adaptation state, gender, level of physical activity, methodology of an experiment and duration of body immersion. Therefore, short-term and repetitive immersion must be distinguished from long-lasting endurance swimming in a cold water due to deep tissue cooling that may cause an impairment of physical and thermoeffector performance (2, 3). Moreover, a large number of studies regarding open cold-water swimming concerns problems related with hypothermia and afterdrop phenomenon using pre- and post-exposure measurements (4, 5), which should not be confused with adaptive changes evoked by repetitive stressor.

It has been shown that regular recreational swimming in a cold water affects human health by initiating immediate and long-term physiological and biochemical responses (6, 7). Evidence-based benefits of cold water swimming and cold water immersion include, among others, improvement of antioxidant protection and immune responses, better recovery of fatigued muscles, analgesia involving different endogenous pathways, and some metabolic improvements (8-13). These positive reactions represent a complex body adaptation to active cold water immersion and are often associated with cold water habituation and acclimatization.

In naked individuals, winter outdoor swimming rapidly decreases body surface temperature because of greater thermal conductance of water and sympathetically driven cutaneous vasoconstriction that occurs early as a natural reflex preventing heat loss. There are also other non-noradrenergic mechanisms that contribute to this reflex (14). At the same time voluntary muscle contractions and brown adipose tissue activity increase

metabolic heat production which, in turn, may lead to skin vasodilation (cold-induced vasodilation, CIVD), rapid muscle cooling and increased oxygen demand for a specific exercise workload reflected by oxygen consumption (VO_2) (15-17). Studying the balance between increased metabolic heat production and the inhibition of heat loss under cold conditions, T. Maeda has found that the peripheral vasomotor system increases sensitivity to cold stress when combined with regular exercise, leading to increased vasoconstriction ability and better cold tolerance (18). Due to initial vasoconstriction cold water exposure limits oxygen delivery to the peripheral tissues (skin and muscles), while physical activity increases body's demands for oxygen. In these circumstances, one may expect body responses in order to sustain oxygen balance between oxygen supply and demand. Level of red blood cells (RBCs) and hemoglobin content reflect body's capacity to carry oxygen in blood. Under normal conditions, any increase in oxygen consumption is associated with an adjustment in oxygen-carrying capacity involving, among others, mechanisms that might reflect increased erythropoietic output. Experimental studies on murine models have shown that significantly increased rate of erythropoiesis and enhanced EPO mRNA expression in the kidney are induced by exposure to low ambient temperature (19). Whether repeated cold water bathing evokes similar effects in humans is unknown. In adults erythropoietin (EPO) is produced by kidneys and, to a lesser degree, by nonhematopoietic sites, including skin (20, 21). Since skin has been proposed to function as an oxygen sensor (22), one may speculate its contribution to the up-regulation of EPO serum levels when potent peripheral vasoconstriction occurs repetitively. Therefore, we raised a hypothesis that regular cold water swimming might enhance the rate of erythropoiesis as a response to transient hypoxia.

Despite a phenomenon referred to as the "exercise paradox", which indicates prothrombotic effects of acute exercise (23), findings from studies have shown that habitual moderate-intensity exercise reduces cardiovascular risk by increasing fibrinolytic activity and stimulating endothelial production of nitric oxide (NO) and prostacyclin (PGI_2), which are potent vasodilators with an anti-thrombotic properties (23-25). Considering the acute effects of moderate exercise and cold water immersion, they are both characterized by higher platelets (PLT) count (26), and, as suggested by others, this increment during acute cold stress can be due to reversal hemoconcentration (27). To date, little is known about the prolonged effect of cold stress-exercise conditioning on resting level of PLT and their activity. Hence, whether regular cold water swimming enhances or inhibits the body's ability to prevent thrombosis remains to be elucidated.

It is agreed that acute and chronic exercise alter the number and function of circulating cells of the immune system. Evidence indicates that acute exercise initially results an increased number of blood neutrophils, monocytes and lymphocytes which is likely due to stress hormones activity and shift from the marginal to circulating pool (28-30). This response is transient, depends on exercise intensity and duration, and reverses soon after exercise session. Moreover, the ability of the immune system to respond to exogenous stimuli may be diminished because mobilized cells may have different functional abilities to those already in the circulation, or, depending on exercise intensity, cortisol may act as a potent immunosuppressive agent (31). According to moderate regular exercise, it has been shown that this type of activity may reduce blood neutrophils and exert long-term anti-inflammatory effects (32, 33). It seems probable that regular cold exposure may have additive effect on boosting immune system because it may induce a physiological change of an adaptive character, associated with increased tolerance to stress. Although there is a large body of anecdotal evidence suggesting that regular ice water bathing positively affects immunity, scientific data are scarce and mixed (34).

In this study we aimed to evaluate body adaptation to physical exercise in a cold environment in order to verify the possible responses in resting blood count level, immunoglobulins and serum erythropoietin concentration in humans.

MATERIALS AND METHODS

Study population

Written agreement of participation was taken from all individuals. The study was performed in accordance with the Declaration of Helsinki and approved by the by the local Ethics Committee (Ethics Committee of Poznan University of Medical Sciences; Ref.KB-1006/13, annex Ref.KB- 889/18).

In 2017, before winter swimming season, cold water swimmers (CWSs) were recruited from Kolobrzeg Walruses Club (KWC). Participant recruitment started with an announcement posted on the KWC website before the winter swimming season. All individuals interested in the study participation were asked to return a declaration form with a baseline questionnaire and contact details. In the second phase of recruitment, individuals were contacted by phone and invited to undergo a medical examination. In October, 72 healthy volunteers regularly practicing outdoor winter swimming for at least 2 years agreed to participate in the study. In January, 62 people applied for further examination and collection of blood (8 were unable to attend without giving any reason, 2 were excluded because of acute infection). In April, 5 people were late for sample collection, 8 were unable to commit time, 1 did not attend because of leg injury, 10 were unable to attend without giving any reason, 4 were excluded because of drinking alcohol the day before. Finally, all three stages of the study were completed by 34 people (16 women and 18 men). To meet inclusion criteria, all participants underwent medical examination and completed assessment survey on general health and physical activity. Diabetes, hypertension, dyslipidemia, inflammation, malignancy, chronic diseases, drug treatment were the factors that disqualified from participating in this study. Exclusion criteria included also any routine medications, hormone replacement therapy, or dietary supplements.

From October to April, twice a week or more than twice a week, CWSs were taking baths in the cold water of the Baltic Sea without wearing protection against the cold. They swam at the natural seawater temperature ranging: 9.5°C in October, 1.0°C in January and 4.4°C at the end of April. Cold water bathing was preceded by a short lasting (few minutes) warm-up activity. CWSs swam at the intensity comparable with recreational swimming (between 3 – 6 metabolic-equivalents) for less than 15 min (most often 5 – 8 min). All study participants were asked to maintain their normal dietary and physical activity behaviors. For examining the possible adaptive effects of cold water swimming fasting blood samples were taken at three time-points of our study: at the beginning (October), in the middle (January) and at the end (April) of one winter swimming season.

The control group (n = 23) was recruited from occupational medicine outpatient clinic among individuals, who practiced recreational walking. Inclusion criteria were similar to the CWS group except from different winter physical activity. We included healthy adults who did not exceed 70% of maximal heart rate (HRmax) when walking at approximately 3 mph for 15 – 30 min twice a week. This type of physical activity represents moderate intensity of exercise (3.0 – 6.0 metabolic equivalent of

task, METs) comparable with recreational swimming. Exercise intensity was controlled using target heart rates (less than 70% of HRmax) and the talk test (ability to talk during exercise). HRmax was calculated by Maximum Heart Rate Calculator (available on Norwegian University of Science and Technology web site: https://www.ntnu.edu/cerg/hrmax), which is based on a formula: 211 – 0.64 × age. Heart rate during exercise was measured using commercially available smart wirstbands (Smartband SAMSUNG Galaxy Fit-e). Initially 30 people were recruited. In January, one of them was excluded because of acute infection and 6 was unable to attend without giving any reason.

Anthropometric and biochemical measurements

Anthropometric parameters included body mass index (BMI). Weight was measured to the nearest 10 g (electronic personal scale, Mensor WE150P1). Height was measured to the nearest 5 mm, using a wall-mounted stadiometer (Comed). Body mass index (BMI; kg/m^2) was calculated as weight (kg)/height (m)^2.

To examine long-lasting adaptive responses and exclude rapid effect of cold stress and physical activity fasting blood samples (following 10 hours of nocturnal fast), before cold swimming, were taken twice a week within the first weeks of October, January and April.

Serum erythropoietin (EPO) was examined by Elisa method (Elisa kit cat. No EIA3646, DRG international, Germany) using Synergy™ 2 Multi-Detection Microplate Reader (Biotek Instruments, Winooski, VT, USA). Dimension EXL with LM Integrated Chemistry System Analyzer combined with Advia 2120 system (Siemens, Newark, US) was used to determine the following parameters: complete blood count (CBC) including evaluation of red blood cells (RBC) count, hemoglobin (Hb), hematocrit (Hct) and red blood cells indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) count with WBC differential, platelets (PLT count) as well as serum folate and serum immunoglobulins (IgG, IgA, IgM).

Statistical analysis

Statistical analysis was performed by Statistica for Windows 10.0 (StatSoft, Poland). Analyzed data were presented as means and standard deviations or medians with a range. The assumptions that data follow a normal distribution were checked by Shapiro-Wilk's test. The t-Student test was used for comparing data with normal distribution. In case the data did not follow a normal distribution differences between groups were detected by Mann-Whitney U-test. One way analysis of variance (ANOVA) repeated measure with Tukey's HSD *post-hoc* test or the Friedman ANOVA with Dunn's *post-hoc* test for variables lacking normal distribution were applied for seasonal changes analysis. P-value below 0.05 was considered as significant.

RESULTS

General health

Among 34 CWSs only 4 individuals experienced mild symptoms of common cold lasting no more than 7 days within whole winter time. In the control group 2 people required antibiotic treatment due to the upper respiratory tract infection

Table 1. Resting variables of CWS and control group at the beginning of the study (October).

Variable	Study population		
	CWS (n = 34)	**CG** (n = 23)	**P** ^{test}
Age (years)	50.0 ± 12.2	46.0 ± 14.1	0.073 ^{TS}
BMI (kg/m^2)	26.7 ± 4.6	25.8 ± 3.4	0.132 ^{TS}
Folate (ng/mL)	10.5 (5.2 – 23.2)	10.8 (6.5 – 13.3)	0.852 ^{MW}
Hb (mmol/L)	8.6 (5.7 – 10.2)	8.7 (7.5 – 9.9)	0.574 ^{MW}
WBC (×10^6/L)	5.6 ± 1.4	6.4 ± 1.5	0.038 ^{TS}
RBC (×10^{12}/L)	4.8 ± 0.4	4.8 ± 0.4	0.947 ^{TS}
Hct (L/L)	0.4 (0.3 – 0.5)	0.4 (0.3 – 0.5)	0.955 ^{MW}
MCV (fL)	90.9 (71.9 – 96.0)	90.9 (71.8 – 94.5)	0.564 ^{MW}
MCH (fmol)	1.8 (1.3 – 2.0)	1.8 (1.3 – 1.9)	0.417 ^{MW}
MCHC (mmol/L)	19.9 (17.8 – 20.8)	19.8 (17.8 – 20.5)	0.187 ^{MW}
PLT (×10^9/L)	249.9 ± 54.4	238.8 ± 51.2	0.443 ^{TS}
Neutrophils (×10^9/L)	2.9 ± 1.2	2.7±1.2	0.566 ^{TS}
Lymphocytes (×10^9/L)	1.5 (1.0 – 3.3)	1.6 (1.0 – 3.3)	0.569 ^{MW}
Monocytes (×10^9/L)	0.3 (0.2 – 0.9)	0.3 (0.2 – 0.7)	0.684 ^{MW}
Eosinophils (×10^9/L)	0.2 (0.0 – 0.5)	0.1 (0.0 – 0.3)	0.353 ^{MW}
Basophils (×10^9/L)	0.0 (0.0 – 0.1)	0.0 (0.0 – 0.1)	0.273 ^{MW}
IgG (g/L)	11.8 ± 2.0	12.2 ± 1.3	0.349 ^{TS}
IgA (g/L)	2.5 ± 1.1	2.9 ± 0.7	0.099 ^{TS}
IgM (g/L)	0.9 (0.3 – 2.3)	1.3 (0.8 – 2.1)	0.018 ^{MW}
EPO (IU/L)	6.3 ± 1.7	6.1 ± 1.6	0.672 ^{TS}

Normally distributed data are presented as means ± SD. Non-normal data are presented as medians. The threshold level of significance is P ≤ 0.05. *Abbreviations*: BMI, body mass index; CWS, cold water swimmers; CG, control group; EPO, erythropoietin; Hb, haemoglobin; Hct, haematocrit; MW, Mann-Whitney test; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; RBC, red blood cells; TS, T-Student test; WBC, white blood cells.

Table 2. Variables of cold water swimmers (n = 34) within one swimming season.

Variable	Swimming season - month			Test		Comparison		
	October (1)	January (2)	April (3)	F	P test	1 vs. 2	1 vs. 3	2 vs. 3
BMI (kg/m^2)	26.7 ± 4.6	26.9 ± 4.7	26.8 ± 4.7	10.43	0.356 An			
Folate (ng/mL)	10.5 (5.2 – 23.2)	8.5 (4.6 – 26.7)	7.4 (4.3 – 23.4)	26.86	< 0.001 Fri	0.013	< 0.001	–
Hb (mmol/L)	8.6 (5.7 – 10.2)	9.1 (5.6 – 10.6)	9.4 (6.8 – 10.7)	32.52	< 0.001 Fri	0.011	< 0.001	0.019
WBC ($\times 10^6$/L)	5.6 ± 1.4	5.7 ± 1.6	5.3 ± 1. 6	1.27	0.286 An			
RBC ($\times 10^{12}$/L)	4.8 ± 0.4	4.7 ± 0.4	5.2 ± 0.4	21.5	< 0.001 An	–	< 0.001	< 0.001
Hct (L/L)	0.4 (0.3 – 0.5)	0.5 (0.3 – 0.5)	0.5 (0.4 – 0.5)	14.06	0.001 Fri	–	0.001	–
MCV (fL)	90.9 (71.9 – 96.0)	94.6 (70.1 – 99.4)	90.3 (79.5 – 94.5)	38.88	< 0.001 Fri	< 0.001	–	< 0.001
MCH (fmol)	1.8 (1.3 – 2.0)	1.9 (1.3 – 2.0)	1.9 (1.6 – 2.0)	45.83	< 0.001 Fri	< 0.001	0.003	0.003
MCHC (mmol/L)	19.9 (17.8 – 20.8)	20.4 (18.3 – 21.9)	20.6 (19.6 – 21.7)	36.41	< 0.001 Fri	< 0.001	< 0.001	–
PLT ($\times 10^9$/L)	249.9 ± 54.4	238.3 ± 52.3	221.6 ± 45.5	5.33	0.007 An	–	0.005	
Neutrophils ($\times 10^9$/L)	2.9 ± 1.2	3.2 ± 1.3	2.9 ± 1.3	5.72	0.005 An	0.009	–	0.020
Lymphocytes ($\times 10^9$/L)	1.5 (1.0 – 3.3)	1.6 (1.0 – 3.3)	1.6 (1.0 – 3.3)	10.23	0.006 Fri	0.001	–	–
Monocytes ($\times 10^9$/L)	0.3 (0.2 – 0.8)	0.4 (0.2 – 0.8)	0.3 (0.2 – 0.8)	2.24	0.327 Fri			
Eosinophils ($\times 10^9$/L)	0.2 (0.0 – 0.5)	0.2 (0.0 – 0.5)	0.2 (0.0 – 0.5)	1.76	0.416 Fri			
Basophils ($\times 10^9$/L)	0.0 (0.0 – 0.1)	0.0 (0.0 – 0.1)	0.1 (0.0 – 0.1)	6.88	0.032 Fri	–	–	0.028
Ig G (g/L)	11.8 ± 2.0	11.3 ± 2.0	10.9 ± 1.9	19.27	< 0.001 An	0.005	< 0.001	0.012
Ig A (g/L)	2.5 (0.7 – 5.0)	2.2 (0.7 – 13.1)	2.2 (0.8 – 4.8)	21.73	< 0.001 Fri	–	< 0.001	0.023
Ig M (g/L)	0.9 (0.3 – 2.3)	0.9 (0.2 – 2.2)	0.8 (0.2 – 2.1)	24.76	< 0.001 Fri	–	< 0.001	0.023
EPO (IU/L)	6.3 ± 1.7	8.8 ± 2.3	8.1 ± 1.9	16.19	< 0.001 Fri	0.005	0.001	–

Normally distributed data are presented as means ± SD. Non-normal data are presented as medians. The threshold level of significance is P ≤ 0.05. *Abbreviations*: 1, variable measured in October; 2, variable measured in January; 3, variable measured in April within one swimming season; An, Anova test; BMI, body mass index; EPO, erythropoietin; Fri, Friedman test; Hb, haemoglobin; Hct, haematocrit; MW, Mann-Whitney test; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; RBC, red blood cells; WBC, white blood cells.

and 5 reported symptoms of common cold with treatment directed at relieving signs.

Comparison of variables measured in cold water swimmers with the control group

The baseline biochemical parameters and demographic characteristics of CWCs and controls are given in *Table 1*. Any statistically significant change in measured parameters did not exceed normal/reference range.

There were no statistical differences between CWSs and the control group in term of BMI (P = 0.132). Both, CWSs and controls were not obese. CWSs had lower WBC count (P = 0.038) and serum IgM concentration (P = 0.018) when compared with the controls. Serum EPO and folate concentrations did not differ significantly when compared with the control group (P = 0.672 and P = 0.852, respectively).

Seasonal changes in serum erythropoietin measured at three time-points within one winter swimming season

In all CWSs serum EPO significantly increased in January, and in April (at first and second time-point) with respect to October (*Table 2*). In female CWSs serum EPO levels were significantly increased in the middle of swimming season (second time-point) and remained at that level until the end of the study (*Table 3*). Similar trend was observed in male CWSs, but the change was not statistically significant (*Table 4*). There were no seasonal changes in serum EPO levels in the control group (*Table 5*).

Table 3. Variables of female cold water swimmers (n = 16) within one swimming season.

Variable	Swimming season - month			Test		Comparison		
	October (1)	**January (2)**	**April (3)**	**F**	**P^{test}**	**1 vs. 2**	**1 vs. 3**	**2 vs. 3**
BMI (kg/m^2)	26.4 ± 6.0	26.6 ± 6.2	26.2 ± 6.0	15.56	0.215 An			
Folate (ng/mL)	8.7 (5.1 – 20.6)	8.0 (4.6 – 18.0)	6.5 (4.2 – 16.7)	9.13	0.010 Fri	–	0.008	-
Hb (mmol/L)	8.2 (5.7 – 8.7)	8.4 (5.8 – 9.7)	8.8 (6.9 – 10.2)	15.71	< 0.001 Fri	–	< 0.001	-
WBC (×10^6/L)	5.5 ± 1.6	5.7 ± 1.8	4.9 ± 1.5	4.61	0.018 An	–	–	0.018
RBC (×10^{12}/L)	4.5 ± 0.2	4.4 ± 0.4	4.7 ± 0.3	11.35	< 0.001 An	–	0.005	< 0.001
Hct (L/L)	0.4 (0.3 – 0.5)	0.4 (0.3 – 0.5)	0.4 (0.4 – 0.4)	4.88	0.087 Fri			
MCV (fL)	91.0 (71.9 – 94.9)	95.6 (70.1 – 98.4)	91.3 (79.5 – 94.3)	14.63	< 0.001 Fri	0.024	–	< 0.001
MCH (fmol)	1.8 (1.3 – 2.0)	2.0 (1.3 – 2.0)	1.9 (1.6 – 2.0)	17.84	< 0.001 Fri	< 0.001	–	-
MCHC (mmol/L)	19.8 (17.8 – 20.8)	20.5 (18.3 – 21.9)	20.6 (19.6 – 21.3)	16.63	< 0.001 Fri	0.008	<0.001	–
PLT (×10^9/L)	257.5 ± 45.0	247.1 ± 55.4	227.7 ±42.8	4.39	0.021 An	–	0.018	–
Neutrophils (×10^9/L)	2.6 ± 1.0	3.1 ± 1.0	2.7 ± 1.0	4.33	0.022 An	0.028	–	–
Lymphocytes (×10^9/L)	1.6 (1.0 – 3.3)	1.7 (1.0 – 3.3)	1.6 (1.0 – 3.3)	3.26	0.196 Fri			
Monocytes (×10^9/L)	0.3 (0.2 – 0.7)	0.4 (0.2 – 0.7)	0.3 (0.2 – 0.7)	3.22	0.200 Fri			
Eosinophils (×10^9/L)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.55	0.585 An			
Basophils (×10^9/L)	0.0 (0 – 0.1)	0.0 (0 – 0.1)	0.0 (0 – 0.1)	2.57	0.276 Fri			
IgG (g/L)	11.7 ± 1.7	10.9 ± 1.4	10.3 ± 1.3	11.67	< 0.001 An	–	<0.001	0.005
IgA (g/L)	2.2 ± 1.1	2.0 ± 0.9	1.9 ± 0.8	2.43	0.105 An			
IgM (g/L)	1.1 ± 0.6	1.1 ± 0.5	1.0 ± 0.5	7.67	0.002 An	–	0.002	0.035
EPO (IU/L)	5.4 (4.0 – 9.6)	8.5 (5.5 – 15.1)	8.7 (5.9 – 12.8)	19.46	< 0.001 Fri	< 0.001	< 0.001	–

Normally distributed data are presented as means ± SD. Non-normal data are presented as medians. The threshold level of significance is P ≤ 0.05. *Abbreviations*: : 1, variable measured in October; 2, variable measured in January; 3, variable measured in April within one swimming season; An, Anova test; BMI, body mass index; EPO, erythropoietin; Fri, Friedman test; Hb, haemoglobin; Hct, haematocrit; MW, Mann-Whitney test; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; RBC, red blood cells; WBC, white blood cells.

Seasonal changes in serum folate measured at three time-points within one winter swimming season

In CWSs serum folate concentrations decreased significantly at second and third time-point of the study, when compared with the beginning (*Table 2*). Statistically significant decrease was present both, in males and females. In the controls, when compared with the baseline values, serum folate level dropped in January and then returned to initial values.

Seasonal changes in other parameters measured at three time-points within one winter swimming season

At the end of swimming season values of Hb, RBC, MCHC and MCH were significantly higher in the CWS group when compared with the beginning of the study (first versus third time point of the study). Conversely, PLT, IgG, IgM and IgA levels were significantly lower (*Table 2*). Considering females and males separately, drop in PLT was seen only in women (*Table 3* and *Table 4*).

In the control group no significant changes were observed in the following parameters: Hb, RBC, WBC, Hct, MCH, MCHC, IgG, IgM, while PLT concentration increased in the second and third time point compared with the baseline values (*Table 5*).

Regarding gender-specific findings in the control group - when compared with baseline values increased resting mean PLT was observed in the mid-season both, in men and women (242.0 ± 59.1 versus 330.0 ± 57.9, P = 0.028 and 235.9 ± 45.1 versus 332.2 ± 73.0, P = 0.020 in men and in women respectively), and this effect persisted after next four months in

Table 4. Variables of male cold water swimmers (n = 18) within one swimming season.

Variable	Swimming season - month			Test		Comparison		
	October (1)	January (2)	April (3)	F	P^{test}	1 vs. 2	1 vs. 3	2 vs. 3
BMI (kg/m^2)	25.4 ± 5.9	25.6 ± 6.2	25.2 ± 6.0	18.57	0.200 An	–	–	–
Folate (ng/mL)	11.5 (7.5 – 23.2)	9.0 (5.6 – 26.7)	8.1 (5.1 – 23.4)	18.85	< 0.001 Fri	0.018	< 0.001	–
Hb (mmol/L)	8.9 (8.1 – 10.2)	9.4 (8.7 – 10.6)	9.8 ± 0.5	16.93	< 0.001 Fri	–	< 0.001	–
WBC ($\times 10^6$/L)	5.4 (3.7 – 8.6)	5.4 (3.9 – 8.1)	5.8 (2.5 – 9.1)	0.11	0.946 Fri			
RBC ($\times 10^{12}$/L)	4.9 (4.7 – 5.7)	4.9 (4.6 – 5.5)	5.4 (4.8 – 5.7)	10.68	0.005 Fri	–	0.047	0.006
Hct (L/L)	0.5 (0.4 – 0.5)	0.5 (0.4 – 0.5)	0.5 (0.4 – 0.5)	9.66	0.008 Fri	–	0.006	–
MCV (fL)	90.6 ± 2.8	94.0 ± 3.0	89.9 ± 3.1	45.00	< 0.001 An	< 0.001	–	< 0.001
MCH (fmol)	1.8 (1.4 – 1.9)	1.9 (1.8 – 2.0)	1.9 (1.8 – 2.0)	28.37	< 0.001 Fri	< 0.001	0.018	0.037
MCHC (mmol/L)	20.1 ± 0.4	20.4 ± 0.5	20.8 ± 0.4	21.77	< 0.001 An	0.015	< 0.001	0.003
PLT ($\times 10^9$/L)	243.2 ± 62.1	230.4 ± 49.5	216.1 ± 48.3	1.86	0.171 An			
Neutrophils ($\times 10^9$/L)	3.1 ± 1.3	3.3 ± 1.4	3.1 ± 1.4	1.51	0.235 An			
Lymphocytes ($\times 10^9$/L)	1.5 (1.2 – 2.9)	1.6 (1.2 – 2.9)	1.6 (1.2 – 2.9)	7.28	0.026 Fri	0.006	–	–
Monocytes ($\times 10^9$/L)	0.4 (0.2 – 0.8)	0.4 (0.2 – 0.8)	0.4 (0.2 – 0.8)	1.14	0.565 Fri			
Eosinophils ($\times 10^9$/L)	0.2 (0.1 – 0.5)	0.2 (0.1 – 0.5)	0.2 (0.1 – 0.5)	2.95	0.229 Fri			
Basophils ($\times 10^9$/L)	0.1 (0.0 – 0.1)	0.1 (0.0 – 0.1)	0.1 (0.0 – 0.1)	4.45	0.108 Fri			
Ig G (g/L)	12.3 ± 2.2	11.7 ± 2.4	11.5 ± 2.1	10.11	< 0.001 An	0.006	< 0.001	–
Ig A (g/L)	2.8 (1.0 – 5.0)	2.8 (0.9 – 13.1)	2.7 (0.9 – 4.8)	16.33	< 0.001 Fri	–	< 0.001	0.037
Ig M (g/L)	0.9 ± 0.5	0.9 ± 0.5	0.8 ± 0.4	3.60	0.062 An			
EPO (IU/L)	6.8 (3.7 – 9.4)	8.5 (5.7 – 13.5)	7.6 (5.8 – 13.9)	2.33	0.311 Fri			

Normally distributed data are presented as means ± SD. Non-normal data are presented as medians. The threshold level of significance is $P \leq 0.05$. *Abbreviations*: 1, variable measured in October; 2, variable measured in January; 3, variable measured in April within one swimming season; An, Anova test; BMI, body mass index; EPO, erythropoietin; Fri, Friedman test; Hb, haemoglobin; Hct, haematocrit; MW, Mann-Whitney test; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; RBC, red blood cells; WBC, white blood cells.

women only (235.9 ± 45.1 in October versus 281.1 ± 65.3 in April, P = 0.03).

No other gender-specific changes regarding controls were observed.

DISCUSSION

Results of the present study demonstrate that 7 months of regular cold water swimming during winter season contribute to increased serum EPO concentration. To our knowledge, this is the first study testing the hypothesis that repeated short-term cold water bathing can influence production of EPO in human organism.

It is widely accepted that exposure to cold water reduces blood flow through the skin and kidneys, which is sustained throughout several minutes after immersion (21, 35, 36). Therefore, we hypothesized that regular and short-lasting winter swimming, that serves as a repetitive stressor working through restricted cutaneous blood flow and transient hypoxia, may exert adaptive changes on erythropoiesis. After three months of regular cold exposure we found higher resting EPO concentrations in CWSs as compared with the initial values. This effect was also present after next four months of regular cold water bathing. Recently, Buemi *et al*. have shown that skin keratocytes can produce EPO as a response to different oxygen concentrations (21). Reduction of blood flow through the skin initiates keratocyte hypoxia which can be essential component of

Table 5. Variables of control group (n = 23) within one swimming season.

Variable	Swimming season - month			Test		Comparison		
	October (1)	January (2)	April (3)	F	P^{test}	1 vs. 2	1 vs. 3	2 vs. 3
BMI (kg/m^2)	25.8 ± 3.4	26.0 ± 3.2	25.9 ± 4.0	7.55	0.250 An			
Folate (ng/mL)	10.7 ± 1.9	9.5 ± 1.8	10.8 ± 1.3	9.50	< 0.001 An	0.002	–	< 0.001
Hb (mmol/L)	8.7 ± 0.5	8.9 ± 0.7	8.9 ± 0.5	4.03	0.255 An			
WBC ($\times 10^6$/L)	6.4 ± 1.5	6.2 ± 1.0	6.2 ± 1.7	2.65	0.675 An			
RBC ($\times 10^{12}$/L)	4.7 (4.3 – 5.7)	4.7 (4.0 – 5.7)	4.7 (4.1 – 5.3)	3.05	0.623 Fri			
Hct (L/L)	0.4 (0.3 – 0.5)	0.4 (0.4 – 0.5)	0.5 (0.4 – 0.5)	0.24	0.180 Fri			
MCV (fL)	90.9 (71.8 – 94.5)	90.2 (82.7 – 92.2)	89.1 (79.9 – 91.6)	18.83	< 0.001 Fri	0.037	< 0.001	–
MCH (fmol)	1.8 (1.3 – 1.9)	1.8 (1.6 – 2.1)	1.8 (1.0 – 2.0)	5.15	0.295 Fri			
MCHC (mmol/L)	19.8 (17.8 – 20.5)	19.7 (18.1 – 20.5)	19.8 (18.3 – 20.5)	1.61	0.309 Fri			
PLT ($\times 10^9$/L)	238.8 ± 51.2	331.3 ± 64.8	294.8 ± 51.9	16.82	< 0.001 An	< 0.001	0.005	–
Neutrophils ($\times 10^9$/L)	2.7 ± 1.2	3.6 ± 1.5	3.2 ± 0.8	0.49	0.033 An	0.026	–	–
Lymphocytes ($\times 10^9$/L)	1.6 (1.0 – 3.3)	1.5 (0.8 – 3.0)	1.9 (1.4 – 3.5)	3.44	0.003 Fri	–	–	0.002
Monocytes ($\times 10^9$/L)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.88	0.355 An			
Eosinophils ($\times 10^9$/L)	0.1 (0.0 – 0.3)	0.1 (0.0 – 0.4)	0.1 (0.1 – 0.5)	0.03	0.792 Fri			
Basophils ($\times 10^9$/L)	0.0 (0.0 – 0.2)	0.0 (0.0 – 0.1)	0.0 (0.0 – 0.1)	2.94	0.184 Fri			
Ig G (g/L)	12.2 ± 1.3	11.9 ± 1.3	12.1 ± 1.3	2.88	0.257 An			
Ig A (g/L)	2.9 ± 0.7	2.5 ± 0.8	3.2 ± 0.7	8.30	0.003 An	–	–	0.002
Ig M (g/L)	1.3 (0.8 – 2.1)	0.9 (0.7 – 1.8)	1.5 (0.8 – 2.0)	3.13	0.062 Fri			
EPO (IU/L)	6.1 ± 1.6	6.2 ± 1.1	6.3 ± 1.5	0.15	0.859 An			

Normally distributed data are presented as means ± SD. Non-normal data are presented as medians. The threshold level of significance is $P \leq 0.05$. *Abbreviations*: 1, variable measured in October; 2, variable measured in January; 3, variable measured in April within one swimming season; An, Anova test; BMI, body mass index; EPO, erythropoietin; Fri, Friedman test; Hb, haemoglobin; Hct, haematocrit; MW, Mann-Whitney test; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; RBC, red blood cells; WBC, white blood cells.

systemic hypoxic response, including the skin-kidney axis - a new pathway in the control of EPO production (21). Hypoxia, *per se* induces several physiological responses, including expression of genes required for glycolysis, cell proliferation, apoptosis and erythropoiesis (37). Recent findings suggest that reduction of blood flow induced by cold immersion can cause skin hypoxia and strongly stimulates expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), one of the major factors regulating mitochondrial biogenesis and cellular energy metabolism (38, 39) PGC-1α increases oxygen consumption, leading to stabilization of hypoxia inducible factor -1 (HIF-1) (40-42), which, in turn, is a pivotal mechanism in the transcriptional activation of EPO gene (43, 44). Thus, PGC-1α and HIF-α appear to be a very important regulatory factors that mediate erythropoiesis under conditions of cold and acute hypoxia (42, 44, 45). The above research seem to support our hypothesis that repetitive and short-term cold exposure may affect EPO production. Interestingly, in our study, more evident changes were present in women, whereas men showed only a tendency to increase EPO levels. Men and women differ in thermoregulatory responses to cold stress. Women cool more rapidly (40) and, therefore, greater cold-induced stressor may evoke more pronounced body's response. Whether exercise and cold exposure act independently or dependently remains unclear. Several training studies have shown that exercise alone causes only small decrease in arterial pO2 which is barely sufficient to induce relevant EPO production. As suggested by Schmidt *et al*. EPO is not directly

influenced neither by maximal nor submaximal exercise, and exercise-induced adrenergic stimulation is not a major stimulus of EPO production in humans (47). Our results are consistent with these findings, because we did not observe any significant EPO changes in physically active controls. Moreover, the two studied groups (CWS and control) were similar in their resting EPO levels at the beginning of the study and exhibited comparable level of physical activity during whole winter season.

After 6 months of regular cold water bathing we found non-pathological (within normal range) changes in blood morphology, including elevated resting values of Hb, RBC, MCHC and decreased PLT. Additionally, we noted significant increments in MCV and MCH. The increase in RBC and RBC indices may partly result from an intensified hematopoiesis induced by cold-stimulated increase in EPO concentration. A number of data demonstrate that cold water swimming changes Hb concentration, Htc level, and elevates counts of RBC (6, 27, 48). The alterations are described as an adaptation to low temperature and hemoconcentration resulting mainly from enhanced diuresis, fluid shift and, consequently, decreased plasma volume (2). On the other hand, there are studies reporting that these changes could reflect intensified hematopoiesis and modification of plasma volume due to enhanced sympathetic nervous system activity (49, 50).

Increased EPO stimulates the survival of more erythroid progenitor cells in the EPO-dependent stages and consequently enhances erythrocyte production (51). Interestingly, we observed that regular cold water swimming is associated with significant folate drop, which, as we suspect, might possibly contribute to this phenomenon. In experiments on rats, Kim *et al*. discovered that 5 weeks of regular aerobic exercise lowered resting plasma folate (52). As they suggested, folate drop was due to the increased folate use with enhanced methylation and regeneration processes. Folate affects pathways critical for the adaptation to physical activity and is required for carrying one-carbon units for DNA synthesis, cell proliferation and also for methylation reactions converting homocysteine to methionine (53). Considering our results, both studied groups represented similar level of physical activity but significant decrease in serum folate with concomitant enhancement in EPO, RBC and RBC indices in the middle and at the end of the study was present only in CWS group. It may indicate serum folate fluctuations related to enhanced erythropoiesis in CWSs during winter swimming season, when natural dietary supply is rather small. Humans depend on dietary intake of folate and serum folate is the earliest, highly responsive to natural supply indicator of altered folate exposure. As reported by others, folate dietary intake in Polish adult population is suboptimal or even low, particularly during winter months (54). This may explain decreased serum folate in both groups in the coldest winter month - January. It, however, should not be the case of reduced folate in CWS group at the end of swimming season. Controls, as they reported, were on similar type of diet, their folate concentrations returned to the initial values with Spring, and the changes in folate in the control group although significant, were small. Whether folate status was changed in CWSs regardless of serum folate concentrations requires further determination of folate content in the circulating RBC because RBC folate values reflect general tissue supply (53).

The next finding of our study presents resting level of PLT at three time points of 7-month swimming season. The control group showed increased resting mean PLT in the mid-season (in January) and this effect persisted after next four months in women only, which may suggest more dynamic changes in female organism. In contrast, CWS showed significant decrease in PLT count in the middle and at the end of winter swimming period suggesting adaptive changes to repeated short-lasting cold exposure. Although it is well recognized that exercise accelerates blood clothing and induces PLT release from spleen, bone marrow and lungs (55), cold stress in combination with exercise may evoke additional thermal responses of the body. Over fifty years ago, Finkel *et al*. demonstrated that increased coagulability and PLT count after exercise are lessened when activity is performed in the cold (56). On the other hand, Lombardi *et al*. have found increased PLT count following single cold water swimming session, when blood drawings were performed the day before and immediately after the performance (49). Considering our results, one of the possible explanatory mechanisms may indicate beginning of cold-induced PLT clearance. Based on the two pathways contributing to basal PLT clearance: one related with local clotting and another with phagocytosis of excessively primed platelets by liver macrophages, Hoffmeister *et al*. described thermosensor properties of PLT (57). According to their findings, PLT become primed for activation at lower temperatures at the peripheral body sites. Repetitive priming predisposes PLT to be recognized by hepatic macrophage complement type 3 (CR3) receptors initiating phagocytosis (57). Hypothermia decreases circulating PLT count and may even provoke thrombocytopenia (58). Depending on tissue insulation, level of physical activity in a cold water, gender and other factors, it takes 20 – 30 minutes for hypothermia to occur. In the present study, CWSs spent less than 15 minutes (on average 5 – 8 min) in the cold sea water and were not hypothermic. Nevertheless, their physical activity just before and during bathing could intensify rapid cooling of skin and muscle during cold exposure, predisposing to repeated PLT priming. Interestingly, this effect was significant only in female swimmers, which may be associated with gender-related response to cold and more dynamic changes in women. Similarly in our previous study, the favorable effect of regular cold water swimming on selected cardiovascular risk factors (blood homocysteine, lipid profile, and ApoB/ApoA-I ratio) was more pronounced in women (59).

Although standard deviations (SDs) for PLT were high, they were fairly similar for both groups (CWS and control) suggesting similar distribution of scores. Clearly, further research is needed to evaluate these assumptions.

Regarding white blood cells examination, we found that CWSs demonstrated lower concentration of WBC and lower levels of general immunoglobulin subpopulations (with statistically significant difference only in IgM), when compared with the controls. Moreover, regular active exposition to cold was associated with statistically significant, within normal range, decrease in all studied classes of immunoglobulins. These observations may indicate lower recruitment of immune cells when organism is not exposed to pathogen, which corresponds to anti-inflammatory effect of cold described by others (60). Moreover, while studying a large array of immune parameters (including WBC and cytokines), Gagnon *et al*. demonstrated reduced exercise-related immune response during exercise under cold conditions compared to a thermoneutral environment (61), while, as shown by Mazur *et al*., 3 months of regular moderate exercise alone has modest regulatory influence on inflammatory markers (62). It is possible that repeated bouts of mild exercise combined with short-lasting cold exposure may induce immune system adaptation. Epidemiological and anecdotal studies show that regular cold water swimming protects from frequent illnesses by boosting an immune system, and improves general wellbeing (63), however, complex and diverse adaptive mechanisms that may enhance the immune responses are still a matter of investigation and require more well controlled studies.

The main strength of this study was the examination, for the first time, of a long-lasting erythropoietic responses to regular

bursts of a mild exercise combined with cold water. The limitations of the current study include relatively small number of study participants. However, recruitment of this specific study group was limited by the number of active members of Kolobrzeg Walruses Club. We admit that reticulocytes were not measured, however, since we investigated longitudinal and final effect of repetitive stressor on erythropoiesis, we were interested in resting values of EPO, Hb and mature RBC over three and seven months of swimming season. Therefore, this could be considered as an arguable weakness of the study. Further research might explore the dynamics of the current observation, including the number and characteristics of reticulocytes, their resting values at the three time points of the study, pre- and post-swimming measurements, and more extensive statistical analysis using two-way ANOVA (in order to detect the effects for time, cold water swimming and the interaction between factors in swimmers and controls). The authors also understand that, in this particular model, the interplay between exercise, cold exposure and adaptive responses in blood elements are complex. Therefore, detailed mechanisms and values of other blood parameters, including immune markers or platelets reactivity, require further investigation. Nevertheless, the authors believe that their findings make several contributions to existing literature and extend the current knowledge of recreational physical activity in extreme thermal conditions.

In summary, the most important finding of this study is that regular and short-lasting cold water swimming induces adaptive changes in the resting blood elements and EPO concentrations. The effect is more evident in female organism. Possibly, these within-normal-range changes prepare an individual to extreme cold conditions *via* better oxygen delivery.

Conflict of interests: None declared.

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