Comparison of Selected Morphological, Rheological and Biochemical Parameters of Winter Swimmers' Blood at the End of One Winter Swimming Season and at the Beginning of Another

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Accepted June 26, 2015

TELEGLÓW A., MARCHEWKA J., TABAROWSKI Z., REMBIASZ K., GŁODZIK J., ŚCISŁOWSKA-CZARNECKA A. 2015. Comparison of selected morphological, rheological and biochemical parameters of winter swimmers' blood at the end of one winter swimming season and at the beginning of another. Folia Biologica (Kraków) **63**: 221-228.

The aim of the study was to examine potential differences in the morphological, rheological and biochemical blood parameters of winter swimmers who remained physically active during the period between the end of one winter swimming season and the beginning of another. The study included a group of healthy winter swimmers (n=17, all between 30 and 60 years of age). Six months following the end of winter season, the levels of mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin turned out to be significantly higher, while erythrocyte count and hematocrit level significantly lower than at the baseline. Moreover, the break in winter swimming was reflected by a significant increase in median erythrocyte elongation index at all shear stress levels ≥ 1.13 Pa. The only significant changes in biochemical parameters of the blood pertained to an increase in the concentration of transferrin and to a decrease in the total protein, albumin and beta-1 globulin concentrations. Seasonal effort of winter swimmers between the end of one winter swimming season and the beginning of another has a positive influence on morphological, rheological and biochemical blood parameters.

Key words: Blood, morphology, rheology, biochemistry, physical activity, winter swimming.

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As recent studies confirmed positive effect of winter swimming on the rheological parameters of the blood (TELEGLOW *et al.* 2014b), we decided to examine changes in the latter parameters taking place beyond the winter season, when the athletes practice other sports disciplines such as jogging, cycling, kayaking, trekking and swimming. The aim of this study was to analyze changes in morphological, rheological and selected biochemical parameters of winter swimmers' blood taking place between the end of one winter swimming season and the beginning of next one (April and November).

Physical activity beside the correct diet is one of basic factors influencing human health. Physical effort plays an important role in the prophylactics of cardiovascular system diseases (WATTS *et al.* 2004). There is considerable evidence concerning the benefits of physical activity for health in adults (KESANIEMI *et al.* 2001). Physical activity also increases vital capacity of the lungs, depth of breathing and oxygen consumption, while decreasing breathing rate and oxygen debt. Moreover, strong respiratory muscles shape chest, leading to its enlargement and improvement of posture, thus considerably enhancing delivery of oxygen to the organism (SCHNOHR et al. 2006). Physical effort also entails favorable changes in the musculoskeletal system: increases both muscle mass and strength, bolsters and stabilizes joints, their range of movement, and prevents their degeneration (ROSEMANN et al. 2008). It also maintains proper bone mineralization (VUORI 1995), prevents and corrects postural defects. Regular physical activity results in positive changes also in relation to the nervous system function: supports intellectual efficiency (ABU-OMAR et al. 2004a), decreases nervous strains, depression and anxiety disorders (HARIS et al. 2006), and mood swings (ABU-OMAR et al. 2004b). The over-season effects of exercise are of importance. FAUDE et al. 2014 describe that four weeks of in-season endurance training can lead to relevant improvements in endurance capacity. TRAN et al. 2014 described changes in physiology, performance, and training practices of elite Australian rowers over 6 months. The paper by MIĄZEK et al. 2005 shows the results of the research on the level of physical activity of 21 years old women lifestyle who study in Kraków. In the group of female students engaging in extra-mural physical activity only 6.7% does it on a daily basis, while regularly 3 times a week -21.4% of them. Once a week practice 45.2%, and out of them 9.4% during weekends. Once a month 3.1%, and 10.2% even less frequently, occasionally during the year. Declaration of seasonal active participation in physical culture was made by 13.4% responders. According to SIEDLIKOWSKA 2012 nordic walking is an ideal way of spending leisure time actively since it not only improves physical condition, but also the well-being of people practicing such a form of activity. Half of participants of nordic walking, devote for this form of movement 4 hours weekly. Some people depending on individual capabilities spend 1, 2, 3, 5 or 6 hours on such activities. Moderate, systematic effort generally has an favorable influence on the immune system by increasing immunity towards infections (ROTHENBACHER et al. 2003).

Being a form of physical effort winter swimming exerts positive effects on health. Apart from an increase in the immunity against infectious diseases, winter swimmers present with improved cardiovascular performance and better perfusion of the skin. Immersion in cold water stimulates significant changes in blood composition, namely an increase in erythrocyte and thrombocyte counts, hematocrit level and hemoglobin concentration, and a decrease in leucocyte count (HOLMER 1974; VOGELAERE *et al.* 1990; TELEGLOW *et al.* 2014a, b). These changes can be explained by a decrease in plasma concentration. According to VOGELAERE et al. (1990), reversal of hemoconcentration observed during and after the cooling processes may be associated with a cold-induced redistribution of body fluids between plasma and tissues. DUGUE & LEPPANEN (2000) showed that regular winter swimmers present with higher resting leukocyte and monocyte counts than the controls.

The aim of this study was to examine potential differences in the morphological, rheological and biochemical blood parameters of winter swimmers who remained physically active during the period between the end of one winter swimming season and the beginning of another one, from April to November.

Material and Methods

Participants

The study included a group of healthy winter swimmers (n=17, all between 30 and 60 years of age) from Kraków 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. Beyond the winter swimming season, i.e. between April and November (6 months), the participants practiced other sports disciplines: jogging, cycling, kayaking, trekking and swimming. Level of their physical activity was estimated using Paffenbarger Physical Activity Questionnaire (PAFFENBARGER 1997) (Table 1). Weekly vigorous activity lasted 1.9 hours, moderate activity 2.1 hours weekly, light activity 7.7 hours weekly, while sitting 3.6 hours, sleeping or reclining 7.7 hours per week. In the group of the studied winter swimmers efforts of such a type and duration lasted for 24 weeks.

Table 1

Median (interquartile ranges) of time activity in 17 males determined at the end of one winter swimming season and at the beginning of another per week using Paffenbarger Physical Activity Questionnaire

Activities	(h)/week
vigorous activity	1.9 (1.5-2.3)
moderate activity	2.1 (1.6-2.7)
light activity	7.7 (6.6-8.9)
sitting activity	3.6 (3.1-4.3)
sleeping or reclining	8.7 (7.1-9.9)

Both the study protocol and all the procedures were approved by the Local Bioethics Committee in Cracow, license no 63/KBL/OIL/2010.

Laboratory procedures

Samples of venous blood collected into the tubes containing EDTA – K3 as an anti-coagulant (8 ml) were obtained from the antecubital vein at the end of one winter swimming season and at the beginning of another one.

Hematological parameters of the blood were determined using automated analyzer ABX MICROS 60 (USA). The following parameters were determined: 1) erythrocyte count $(10^{12}/l)$, 2) hemoglobin concentration (g/l), 3) hematocrit level (l/l), 4) mean corpuscular hemoglobin (MCH, fmol), 5) mean corpuscular volume (MCV, fL), 6) mean cell hemoglobin concentration (MCHC, mmol/l), 7) leukocyte count ($10^9/l$), and 8) thrombocyte count ($10^9/l$).

The aggregation of erythrocytes was determined using a Laser-assisted Optical Rotational Cell Analyzer (LORCA, RR Mechatronics, Holland), as described by HARDEMAN et al. (1994, 2001). 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 $(T\frac{1}{2}, s)$ which describes the kinetics of the aggregation process and is proportional to the time of re-aggregation of disintegrated erythrocyte complexes. Measurements of the aggregation parameters were carried out on a native hematocrit. The temperature in the LORCA was adjusted to 37°C, while all other procedures and measurements were carried out at an ambient temperature ($22\pm1^{\circ}C$). The measurement of the aggregation parameters was based on the detection of laser backscattering from the sheared (disaggregated) and non-sheared (aggregated) blood using a computer-assisted system. Each 2-ml blood sample was transferred to a glass vessel and oxygenated for 10-15 min prior to the measurement. A 1-ml sample of the blood was injected into the gap between the outer cylinder ("cup") and inner cylinder ("bob") of the LORCA. During the measurement, the cup was driven by a computercontrolled 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 lesser light backscattered from aggregating erythrocytes.

Blood glucose concentrations were determined with a stripe method using an Optium Xido device (Abbott Laboratories, Poland). The results were expressed in mmol/l. Concentrations of fibrinogen were determined with a Chrom 7 coagulometer (Slamed Ing GmbH, Germany), with results displayed in g/l.

Statistical analysis

Continuous variables were presented as median and interquartile ranges. The normality of distribution was tested using the Shapiro-Wilk test. The Wilcoxon signed-rank test was used for intergroup comparisons. $SS_{1/2}$ and EI_{max} were calculated by fitting SS versus EI to equation representing Lineweaver-Burke model, using a non-linear curve-fitting algorithm available in a commercial statistical package (Prism 6.2, GraphPad Software Inc., La Jolla, CA). $SS_{1/2}$, EI_{max} and $SS_{1/2}$ to EI_{max} ratio were reported as means \pm standard deviations (SD). The methodology of analysis was previously described in detail (BASKURT & MEISELMAN 2004, 2013; BASKURT et al. 2009). Statistical significance was defined as $P \le 0.05$. All calculations except SS_{1/2} and EI_{max} were performed using Statistica 10 (StatSoft[®], USA) software.

Results

Six months following the end of winter season, the levels of MCHC and MCH turned out to be significantly higher, while erythrocyte count and hematocrit level significantly lower than at the baseline. In contrast, we did not find significant changes in the median leukocyte and thrombocyte counts, hemoglobin concentration and MCV. Similarly, we did not document significant changes in the median levels of glucose and fibrinogen outside the winter swimming season (Table 2).

The break in winter swimming was reflected by a significant increase in median EI at all shear stress levels ≥ 1.13 Pa (Table 3), and a decrease in median SS_{1/2}, EI_{max} and SS_{1/2}/EI_{max} ratio (Table 4).

We did not document significant differences between the median values of aggregation indices determined at the end of one winter swimming season and at the beginning of another (Table 5). Moreover, no significant changes were noted in the median values of most analyzed biochemical parameters of the blood: total serum iron, sTfR, alpha-1 globulin, alpha-2 globulin, beta-2 globulin, gamma globulin, A/G, IgG and CRP. The only significant changes documented during a 6-month break in winter swimming pertained to an increase in the concentration of transferrin and to a decrease in the total protein, albumin and beta-1 globulin concentrations (Table 6).

Table 2

beginning of another			
Parameter	After the season (April)	Before the season (November)	Р
RBC (10 ¹² /l)	4.89 (4.67-5.33)	4.71 (4.37-4.97)	0.002
Hb (g/l)	14.8 (14.4-15.3)	14.4 (14.2-15.1)	ns
Ht (1/1)	45.7 (44.6-47.3)	42.6 (41.6-44.5)	0.001
MCHC (mmol/l)	32.4 (32.2-32.7)	34.0 (33.7-34.2)	< 0.001
MCH (fmol)	30.2 (29.0-30.8)	31.3 (30.5-31.9)	0.002
MCV (fL)	92.5 (90.0-95.0)	93.0 (90.0-94.0)	ns
WBC (10 ⁹ /l)	4.85 (4.3-6.3)	5.2 (4.0-6.4)	ns
PLT (10 ⁹ /l)	209.5 (188.0-237.0)	198 (196.0-235.0)	ns
Glucose (mmol/l)	106.0 (103.0-114.0)	105 (95.0-112.0)	ns
Fibrinogen (g/l)	4.35 (4.12-5.10)	4.64 (4.42-5.10)	ns

Median (interquartile ranges) of basic hematological parameters, and glucose and fibrinogen concentrations in 17 males determined at the end of one winter swimming season and at the beginning of another

ns - non-significant (P>0.05)

Table 3

Median (interquartile ranges) of elongation index (EI) determined at various shear stress in 17 males examined at the end of one winter swimming season and at the beginning of another

Shear stress (Pa)	After the season (April)	Before the season (November)	Р
1.13	0.072 (0.062-0.079)	0.177 (0.166-0.185)	< 0.001
2.19	0.117 (0.108-0.125)	0.290 (0.276-0.300)	< 0.001
4.24	0.211 (0.199-0.222)	0.399 (0.389-0.408)	< 0.001
8.23	0.309 (0.304-0.322)	0.486 (0.482-0.495)	< 0.001
15.96	0.390 (0.386-0.402)	0.545 (0.543-0.551)	< 0.001
31.04	0.461 (0.454-0.476)	0.591 (0.590-0.597)	< 0.001
59.97	0.519 (0.511-0.531)	0.625 (0.622-0.630)	< 0.001

Table 4

Mean \pm standard deviation (SD) of SS_{1/2}, EI_{max} and SS_{1/2}/EI_{max} ratio in 17 males examined at the end of one winter swimming season and at the beginning of another

Parameter	After the season (April)	Before the season (November)	Р
$SS_{1/2}$	6.827 ± 0.924	2.964 ± 0.318	< 0.001
EI _{max}	0.569 ± 0.024	0.654 ± 0.009	< 0.001
SS _{1/2} /EI _{max}	11.999 ± 1.425	4.525 ± 0.451	< 0.001

Table 5

Median (interquartile ranges) of blood rheological parameters in 17 males examined at the end of one winter swimming season and at the beginning of another

Parameter	After the season (April)	Before the season (November)	Р
AI (%)	64.62 (58.03-69.36)	66.67 (62.52-72.56)	ns
T" (s)	2.03 (1.49-2.71)	1.82 (1.29-2.29)	ns
AMP (au)	18.04 (16.17-20.13)	17.46 (14.51-21.68)	ns

Table 6

at the end of one winter swimming season and at the beginning of another			
Parameter	Before the season (April)	After the season (November)	Р
Total serum iron (μ g/dl)	25.21 (14.77-34.30)	21.65 (15.05-25.75)	ns
sTfR (mg/l)	3.2 (2.6-3.3)	2.9 (2.6-3.1)	ns
Transferrin (µg/dl)	2.55 (2.28-2.78)	2.92 (2.49-3.16)	0.002
Total protein (g/l)	80.55 (77.10-81.80)	74.65 (73.60-76.65)	0.001
Albumin (g/l)	48.90 (46.70-51.60)	45.70 (43.50-46.95)	0.001
Alpha-1 globulin (g/l)	2.95 (2.70-3.10)	2.80 (2.70-3.30)	ns
Alpha-2 globulin (g/l)	6.65 (6.10-7.20)	6.50 (6.00-6.85)	ns
Beta-1 globulin (g/l)	4.80 (4.50-5.20)	4.50 (4.20-4.60)	0.004
Beta-2 globulin (g/l)	4.20 (3.80-4.70)	3.80 (3.10-4.45)	ns
Gamma globulin (g/l)	12.40 (11.20-13.30)	11.70 (10.900-12.65)	ns
A/G (g/l)	1.590 (1.490-1.650)	1.585 (1.415-1.695)	ns
IgG (g/l)	12.45 (11.30-13.50)	12.75 (11.60-13.35)	ns
CRP (mg/l)	0.81 (0.43-1.46)	1.10 (0.39-2.08)	ns

Median (interquartile ranges) of biochemical parameters of the blood in 17 males examined at the end of one winter swimming season and at the beginning of another

ns-non-significant (P>0.05)

Discussion

Studies carried out within the scope of the present study were intended to answer the question how the summer brake lasting 6 months affects the organism of winter swimmers especially in respect to blood morphology, and its rheological and biochemical changes. In this period a group of winter swimmers practiced other sports disciplines such as jogging, cycling, kayaking, trekking and swimming.

It is known that swimming is a good aerobic exercise with documented beneficial health effects. Our previous research showed that swimming in cold water induces additional positive changes in erythrocyte deformability (TELEGLOW et al. 2014b), namely an increase in elongation index at shear stress ≥ 1.13 Pa, without concomitant changes in the aggregation indices and plasma viscosity. Deformability is vital for the passage of erythrocytes through a narrow capillary bed and the reduction of blood viscosity under a high shear rates. Deformability of erythrocytes is determined by their structure (protein cytoskeleton, composition and physical properties of the lipid bilayer, and hemoglobin concentration) and surface/volume ratio. Rheological properties of the blood reflect mechanical properties of the plasma and cellular component, as well as the relative proportion of these two phases (i.e. hematocrit level). Plasma, the liquid phase of the blood, is a Newtonian fluid whose viscosity is independent of shear rate. Erythrocytes are the most numerous blood cells, and hence constitute the principal determinant of the cellular phase properties. Hematocrit exerts the strongest effect on blood viscosity and thus any increment above the physiological limit of this parameter results in an exponential increase in blood viscosity. According to COKELET & MEISELMAN (2007), the size of erythrocyte aggregate correlates strongly with plasma fibrinogen concentration. Therefore, the aggregate size is significantly greater in the case of inflammatory conditions leading to acute phase reactions (BASKURT et al. 2011). Previous studies showed that physical exercise leads to acute dehydration, resulting in a decrease in plasma volume, increase in hematocrit level (YALCIN et al. 2003), and impairment of erythrocyte deformability (ERNST et al. 1991) and aggregation (BRUN et al. 2002; GAUDARD et al. 2002). Assuming unchanged hematocrit level, an increase in high shear blood viscosity should be proportional to the rise of plasma viscosity. Low shear blood viscosity is elevated due to an increase in the plasma viscosity and enhanced erythrocyte aggregation. ROMAIN et al. (2011) showed that regular exercise is reflected by a decrease in hematocrit level and erythrocyte aggregation.

Winter swimming stimulates an increase in erythrocyte, leukocyte and thrombocyte counts, hemoglobin concentration and hematocrit level (HOLMER 1974; VOGELAERE *et al.* 1990; LOM-BARDI *et al.* 2011). These changes likely reflect adaptation to low temperature of the water, and hemoconcentration resulting from an enhanced diuresis, leading to a decrease in plasma volume. Hematological parameters of winter swimmers were studied by LOMBARDI *et al.* (2011). They

showed that winter swimming is reflected by changes in the complete blood count, specifically an increase in the erythrocyte, leukocyte and thrombocyte counts. In contrast, they did not observe significant variations in the values of erythrocyte indices, such as MCH, MCV and MCHC (LOMBARDI et al. 2011). Also BAKOVIC et al. (2003) demonstrated that immersion in water (12°C) results in an increase in erythrocyte, leukocyte and thrombocyte counts, and interpreted this phenomenon as an adaptation to exercise performed in cold water. LUKASKI et al. (1990) did not document significant changes in the concentration of hemoglobin and hematocrit level of winter swimmers, but observed an increase in the level of iron metabolism marker, ferritin. LOMBARDI et al. (2011) studied the influence of cold water baths on hematological parameters. A total of 15 volunteers (13 men and 2 women) participated in the experiment that consisted of swimming 150 m in a cold water (6° C). The short-term exposition to cold (whole body immersion in cold water) induced pronounced albeit non-pathological changes in hematological parameters. Cold water baths resulted in an increase in erythrocyte (by 4.7%), thrombocyte (by 25.0%) and leukocyte counts (by 40.6%). Detailed analysis of changes taking place within the leukocyte fraction showed a significant decrease in eosinophil count, along with an increase in the counts of neutrophils (by 42.6%), lymphocytes (by 58.2%) and monocytes (by 27.5%). No changes were noted in mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). The abovementioned changes might result from an intensified hematopoiesis and concomitant decrease in plasma volume caused by a shift of plasma water from the intravascular to the extravascular space due to the sympathetic nervous system activation (LOMBARDI et al. 2011). LUBKOWSKA et al. (2013) found significant changes in hemoglobin concentration, erythrocyte count, hematocrit level, MCV, and percentages of monocytes and granulocytes of swimmers examined after the winter swimming season. The response to cryogenic temperatures was milder after five months of winter-swimming. This points to likely positive adaptive changes in the antioxidant system of healthy winter-swimmers (LUBKOWSKA et al. 2013).

The degree and direction of changes in the blood morphology are determined by the characteristics of physical exercise (type, load, duration and number of repetitions) and the athlete's characteristics (age, sex, training experience) (KELLY 2005 *et al.*, KOCHANSKA-DZIUROWICZ 2007 *et al.*, ROMAIN 2011; MAIRBÄURL 2013). Erythrocyte and leukocyte counts reflect the changes in plasma volume and immunological processes, respectively. Blood obtained immediately after physical exercise is characterized by increased hematocrit level, erythrocyte count and hemoglobin concentration. These changes are interpreted as a consequence of dehydration and resultant hemoconcentration.

A highly increased Hct increases blood viscosity and increases the workload of the heart (EL-SAYED *et al.*, 2005; BÖNING *et al.* 2011). It therefore bears the risk of cardiac overload. An increase in hematocrit due to catecholamineinduced sequestration of red blood cells from spleen is unlikely in humans but has been found in other species (STEWART & MCKENZIE 2002). The magnitude of Hct change seems to depend on exercise intensity during training sessions and the type of exercise (strength vs. endurance; for review see HU & LIN, 2012). A few weeks after the training intervention a new steady state had established, and Hct had returned to pre-training values (SAWKA *et al.* 2000).

There appear to be quite large seasonal variations in Hct (relative change up to 15%) with lower values in summer than in winter that might result in season-to-season changes from $\sim 42\%$ in summer and 48% in winter as fund among several thousand study participants.

We showed that a 6-month break in winter swimming in favor of other physical activities results in a decrease in erythrocyte count and hematocrit level. Probably, these changes reflected the lack of regular physical effort or stress associated with cold water immersion. Other types of physical activity undertaken beyond the winter season resulted in an increase in the two erythrocyte indices, MCH and MCHC. According to the literature, regular physical activity may lead to an increase in MCV, MCH and MCHC (MUJIKA et al. 1998). In contrast, these parameters were shown to remain unchanged after a single swimming session (COR-DOVA et al. 1993), exposure at 0°C at rest or during maximal and submaximal exercise (VOGELAERE et al. 1990). Furthermore, a decrease in MCHC was reported after the whole winter swimming season (TELEGLOW et al. 2014b). Erythrocyte deformability plays an important role in blood flow dynamics, both during and beyond the winter swimming season. The deformability of erythrocytes depends on their cytoplasmic viscosity (i.e. intracellular hemoglobin concentration), resting shape (biconcave disc or alterations thereof) determining membrane surface area to cell volume ratio, and the viscoelastic properties of the cell membrane. Normal human erythrocytes are quite deformable due to their low cytoplasmic viscosity, excess surface area in relation to volume, and the ability of their membranes to freely rotate around

the cytoplasm (i.e. the so-called "tank-treading"). Changes in any of these physical properties may impair the deformability of erythrocytes. $SS_{1/2}$ is a measure of erythrocyte deformability and EI_{max} depicts limiting elongation index at infinite shear stress. EI_{max} is determined by erythrocyte shape and geometry, as well as by its membrane properties. Changes in any of these parameters may lead to a decrease in EI_{max} values.

In this study, erythrocyte deformability determined at a ≥ 1.13 Pa shear stress turned out to be significantly higher after a 6-month break in winter swimming than at the end of the winter swimming season. Together with a significant decrease in $SS_{1/2}$, EI_{max} and $SS_{1/2}/EI_{max}$ ratio, this finding corresponds to a greater deformability of erythrocytes beyond the winter swimming season. This phenomenon is difficult to explain. Perhaps it resulted from the fact that our winter swimmers were well-trained or have been actively involved in other sports disciplines, such as jogging, cycling, kayaking, trekking and swimming both of them may affect deformability of erythrocytes at ≥ 1.13 Pa. FRÖHLICH et al. (1997) indicated that RBC deformability undergoes large seasonal changes, with the peak level in November (14.5 AU) and the lowest level in April (13.1 AU) (P<001).

According to STOCKS *et al.* (2004), winter swimming does not exert any effect on plasma osmolality, total serum protein and electrolyte levels, which suggests that immersion in cold water is associated with a shift of extracellular fluid to interstitial space. FRÖHLICH *et al.* 1997 shown seasonal changes in fibrinogen where maximum level was reached in April, and the seasonal difference was 0,32 g/L.

However, we showed that the break in winter swimming was reflected by a decrease in total protein, albumin and beta 1-globulin concentrations. Consequently, these changes, as well as an increase in the level of transferrin, a protein that transports iron ions in the blood and delivers them to the cells, may be considered a response to the lack of cold water exposure.

The results of this study provide an insight into the changes of hemorheological properties of the blood of athletes during the break between the end of one winter swimming season and the beginning of another.

Acknowledgments

The authors would like to thank the winter swimmers from Kraków Society of Winter Swimmers "Kaloryfer".

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