

## Changes in Biochemical Properties of the Blood in Winter Swimmers\*

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Accepted September 15, 2016

Published December 2016

TELEGLÓW A., MARCHEWKA J., MARCHEWKA A., KULPA J. 2016. Changes in biochemical properties of the blood in winter swimmers. *Folia Biologica (Kraków)* **64**: 285-290.

The aim of the study was to investigate the effects of winter swimming on biochemical indicators of the blood. The subjects – winter swimmers – belonged to the Krakow Walrus Club “Kaloryfer” – “The Heater”. The study group consisted of 11 men, aged 30-50 years, ‘walrusing’ throughout the whole season from November to March. Statistically significant changes throughout the ‘walrusing’ season were observed for the following biochemical parameters: a decrease in sodium (mmol/l), chloride (mmol/l), alpha-2 globulin (g/l), gamma globulin (g/l), IgG (g/l), and an increase in albumin (g/l), indicator A/G, IgA (g/l), Herpes simplex virus IgM. Seasonal effort of winter swimmers has a positive influence on biochemical blood parameters.

Key words: Blood, biochemistry, winter swimming.

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Swimming in cold water, called winter swimming, is a commonly known physical activity. Exposure to cold water causes a number of physiological responses, the purpose of which is to protect the human body against excessive heat loss. Studies have shown that regularly swimming in cold water brings beneficial effects in the physical and mental spheres of man (TELEGLÓW *et al.* 2014; NIEDOSZYTKO *et al.* 2009; KOLETTIS *et al.* 2003; DUGUE & LEPPANEN 2000).

Research has shown that exercise in water at low temperatures stimulates metabolism. Carbohydrates and free fatty acids are the main substrates of energy, there is a decrease in diffusion of lactate in the blood, thanks to which patients regularly bathing in water at cryogenic temperatures are characterized by better adaptation to physical exercise. Furthermore, increased maximal oxygen uptake, achieved at a lower heart rate and, consequently, a shift in anaerobic threshold towards higher exercise loads, can be observed. Protection against excessive heat loss is also evidenced in the vascularization of the areas mostly at risk of too much heat loss, such as around the feet, hands, the

ears and head (ZEMAN 2005; ŻEBROWSKA & POKORA 2005; HUTTUNEN *et al.* 2004; SIEMS *et al.* 1994). The research by NIEDOSZYTKO *et al.* (2009), conducted among Gdansk winter swimmers, showed that people regularly bathing in cold water are less likely to have problems with infections of the upper respiratory tract, characterized by greater exercise capacity, as well as lower risk of cardiovascular system diseases compared to the general population. Those actively participating in walrus clubs are characterized by agreeableness, conscientiousness, they have no difficulty in adapting to the environment and also have a greater ability to constructively deal with stress. In addition, these are people who are social, they have an optimistic outlook on the world and are in search of sensations and experiences.

Application of cold water immersion to subjects with exercise induced muscle injury who had increased plasma levels of myoglobin and creatine kinase (CK) (BAILEY *et al.* 2007). Cold water immersion can also improve antioxidant protection (SIEMS *et al.* 1999). Results presented by GOODALL and HOWATSON (2008) concluded that cold

\*Supported by the Ministry of Science and Higher Education, grant no 73/BS/KRK/2015.

water immersions are not efficient in muscle generation following eccentric exercise. No changes were observed in the values of the tested parameters, i.e. creatine kinase activity (CK), muscle soreness or maximal voluntary contraction (MCV).

The aim of the study was to investigate the effects of winter swimming on the biochemical parameters of the blood in Walruses exposed to changes in ambient temperature. In order to examine the biochemical indicators of the blood, samples of venous blood were taken twice and assayed for electrolytes (sodium, potassium, chloride) and kidney function tests were performed (urea, creatinine, EGFR, uric acid), as well as liver function tests (total bilirubin, AST, ALT, GGT, LDH), electrophoresis (total protein, albumin, alpha-1 globulin, alpha-2 globulin, gamma globulin, A/G indicator, the levels of immunoglobulin (IgG, IgA, IgM) and cortisol, creatine kinase, CRP and the Herpes simplex virus IgM.

## Material and Methods

### Description of the study group

Winter swimmers take winter baths when the water temperature ranges from 1°C to 4°C. The participants belonged to the Krakow Walrus Club “Kaloryfer” – “The Heater”. In order to investigate changes in the biochemical blood properties of people participating in the study, venous blood was collected and tested twice. The samples were taken from the same 11 participants at the beginning and end of the study. The study took place at the Bagry lagoon, at the beginning of the season in November 2015 and at the end of the season, in March 2016. The study group consisted of 13 men aged 30-50 years, ‘walrusing’ throughout the season from November to March.

From the people involved in the study, samples of fasting blood were taken in the morning before going into the water, in the amount of 5 ml from the elbow vein into tubes with clotting accelerators in order to obtain serum. The blood was collected by a qualified nurse under medical supervision. After collection, the blood was transported to the Maria Skłodowska-Curie Memorial Institute – Center of Oncology in Krakow and to the Department of Laboratory Diagnostics – “Diagnostyka” in Krakow. The study was authorized by the Bioethics Committee at the Regional Medical Chamber in Krakow, license no 63/KBL/OIL/2010.

### Biochemical assays

Blood samples were collected in accordance with applicable standards. The serum concentra-

tions of sodium (mmol/l), potassium (mmol/l) and chloride (mmol/l) were determined using the indirect potentiometric method, the levels of urea (mmol/l), creatinine ( $\mu\text{mol/l}$ ), uric acid (mmol/l), total protein (g/l), total bilirubin ( $\mu\text{mol/l}$ ), AST (Unit/l) (aspartate aminotransferase), ALT (Unit/l) (alanine aminotransferase), GGT (Unit/l) (gamma glutamyltransferase), LDH (Unit/l) (lactate dehydrogenase) and CK (Unit/l) (creatine kinase) in the serum were assessed using the spectrophotometric method with a reagent kit and the Roche Diagnostics c501 Cobas analyzer. For all the participants, based on creatinine concentration ( $\mu\text{mol/l}$ ) in the serum, the value of the glomerular filtration rate (eGFR) was estimated based on the MDRD formula (Modification of Diet in Renal Disease Study). CRP concentration (mg/l) (acute phase protein) and immunoglobulin classes: IgG (g/l), IgA (g/l), IgM (g/l), were determined using the immuno-nephelometric method (reagent kits and BN ProSpecNephelometer-Siemens Healthcare Diagnostics). Albumin concentrations (g/l) and globulin fractions (g/l) were calculated on the basis of total protein concentrations (g/l) and the separation of serum proteins using the capillary electrophoresis method with the Sebia Minicap analyzer (Horiba ABX). Cortisol testing (nmol/l) was performed using the ECLIA method with reagent kits and the Roche Diagnostics E411 Cobas analyzer. Determination of the Herpes Simplex Virus (HSV-1/2) was done using the Euroimmun kit.

### Statistical analysis

Continuous variables are presented as mean  $\pm$  standard deviation ( $\bar{x} \pm \text{SD}$ ). The normality of distribution was tested using the Shapiro-Wilk test. Depending on the normality of distribution, intergroup comparisons were performed using the Student's t-test for paired samples or the Wilcoxon signed-rank test. Statistical significance was defined as  $P \leq 0.05$ . All calculations were performed using the Statistica 12 (StatSoft<sup>®</sup>, USA) software.

## Results

**Electrolytes.** We found a statistically significant decrease of sodium (by 2.13%) and chloride levels (by 3.29%) between the beginning and end of the cold water swimming season. A downward trend of potassium level was observed, but without statistical significance (Table 1).

**Renal function tests.** We found no statistically significant changes in creatinine, eGFR and uric

acid levels as presented in Table 2. We observed a downward tendency of plasma urea, but without statistical significance.

Liver function tests. Measured liver tests such as total total bilirubin, AST – aspartate transaminase, ALT – alanine transaminase, GGT – gamma glutamyltransferase and LDH – lactate dehydrogenase showed no statistically significant changes (Table 3).

Serum proteins, cortisol, creatinine kinase. Throughout the cold water swimming season, we found a statistically significant decrease of gamma globulin (by 6.59%), alpha-2 globulin (by 8.49%) and IgG (by 6.75%) levels. We observed a significant increase of albumin (by 4.16%), A/G ratio (by 13.64%), IgA level (by 14.08%) and HSV IgM (by 45%) following winter swimming. We found no statistically significant changes in cortisol or creatinine kinase levels (Table 4 and 5).

Table 1

Selected electrolyte changes between the beginning and end of the swimming season in the group of 11 subjects. Na – sodium, K – potassium, Cl – chloride,  $\bar{x} \pm SD$  – mean value  $\pm$  standard deviation

Parameter	Before swimming season $\bar{x} \pm SD$	After swimming season $\bar{x} \pm SD$	P
Na (mmol/l)	143.33 $\pm$ 2.23	140.27 $\pm$ 2.24*	0.003
K (mmol/l)	4.56 $\pm$ 0.40	4.41 $\pm$ 0.33	0.091
Cl (mmol/l)	100.03 $\pm$ 1.81	96.76 $\pm$ 1.87*	0.00004

\* – statistically significant differences at  $P \leq 0.05$ .

Table 2

Mean values  $\pm$  standard deviations of selected renal function parameters in the group of 11 subjects examined at the beginning and end of the swimming season. eGFR – estimated glomerular filtration rate

Parameter	Before swimming season $\bar{x} \pm SD$	After swimming season $\bar{x} \pm SD$	P
Urea (mmol/l)	5.87 $\pm$ 1.09	5.42 $\pm$ 1.32	0.068
Creatinine ( $\mu\text{mol/l}$ )	84.21 $\pm$ 10.19	83.35 $\pm$ 8.22	0.756
eGFR ( $\text{ml/min/1.73m}^2$ )	83.25 $\pm$ 7.39	75.82 $\pm$ 25.87	0.834
Uric acid ( $\mu\text{mol/l}$ )	342.05 $\pm$ 56.62	339.29 $\pm$ 67.66	0.802

Table 3

Mean values  $\pm$  standard deviations of selected liver function parameters in the group of 11 subjects examined at the beginning and end of the swimming season. AST – aspartate transaminase, ALT – alanine transaminase, GGT – gamma glutamyltransferase, LDH – lactate dehydrogenase

Parameter	Before swimming season $\bar{x} \pm SD$	After swimming season $\bar{x} \pm SD$	P
Total bilirubin ( $\mu\text{mol/l}$ )	13.74 $\pm$ 6.54	15.15 $\pm$ 6.91	0.657
AST (U/l)	26.03 $\pm$ 4.87	23.19 $\pm$ 4.52	0.145
ALT (U/l)	28.54 $\pm$ 13.41	27.09 $\pm$ 14.96	0.859
GGT (U/l)	30.54 $\pm$ 11.31	29.80 $\pm$ 18.67	0.328
LDH (U/l)	185.25 $\pm$ 20.10	174.47 $\pm$ 18.70	0.286

Table 4

Mean values  $\pm$  standard deviations of selected serum protein parameters in the group of 11 subjects examined at the beginning and end of the swimming season. A/G – albumin to globulin ratio

Parameter	Before swimming season $\bar{x} \pm SD$	After swimming season $\bar{x} \pm SD$	P
Total protein (g/l)	76.90 $\pm$ 2.63	76.31 $\pm$ 3.60	0.717
Albumin (g/l)	46.53 $\pm$ 1.39	48.47 $\pm$ 2.75*	0.010
Alpha-1 globulin (g/l)	2.75 $\pm$ 0.35	2.59 $\pm$ 0.39	0.154
Alpha-2 globulin (g/l)	6.83 $\pm$ 1.05	6.25 $\pm$ 1.13*	0.021
Gamma globulin (g/l)	11.83 $\pm$ 1.38	11.05 $\pm$ 1.6*	0.025
A/G	1.54 $\pm$ 0.10	1.75 $\pm$ 0.16*	0.003
IgG (g/l)	12.73 $\pm$ 1.55	11.87 $\pm$ 1.65*	0.001
IgA (g/l)	2.13 $\pm$ 0.50	2.43 $\pm$ 0.71*	0.006
IgM (g/l)	0.98 $\pm$ 0.40	0.96 $\pm$ 0.49	0.715

\* – statistically significant differences at  $P \leq 0.05$

Table 5

Mean values  $\pm$  standard deviations of selected parameters in the group of 11 subjects examined at the beginning and end of the swimming season. CK – creatine kinase, HSV – herpes simplex virus.

Parameter	Before swimming season $\bar{x} \pm SD$	After swimming season $\bar{x} \pm SD$	P
CK (U/l)	195.38 $\pm$ 63.40	188.95 $\pm$ 75.71	0.548
Serum cortisol (nmol/l)	379.14 $\pm$ 91.72	377.23 $\pm$ 159.02	0.670
HSV IgM	0.20 $\pm$ 0.06	0.29 $\pm$ 0.08*	0.011

\* – statistically significant differences at  $P \leq 0.05$ .

## Discussion

In this study we asked – what is the effect of regular winter swimming over a period of 5 months on biochemical indicators of the blood in winter swimmers? We found that systematic winter swimming has an impact on several biochemical and immune system parameters.

### Electrolytes

The observed substantial decrease in sodium and chloride levels is possibly caused by several mechanisms. The most probable explanation of this phenomenon is diuretic-urine sodium loss. Increased diuresis after swimming leads to greater electrolyte loss with the urine. Cold-induced diuresis is caused by central redistribution of blood due to peripheral vasoconstriction and increased hydrostatic pressure (TIPTON & BRADFORD 2014). Circulated plasma volume may be reduced by 24% as described by (MARTINEAU & JACOBS 1988). Other probable mechanisms that may strengthen the above mentioned effect include: higher albumin levels as observed in our study and lower levels of glucocorticoid hormones. We acknowledge that the registered decrease of sodium is in the normal range. However, the lowering of sodium levels within the normal limit called hyponatremia may cause many detrimental effects such as nausea, vomiting, headache, short-term memory loss, confusion, fatigue, loss of appetite, irritability, muscle weakness, spasms or cramps, seizures, decreased consciousness or coma that may be harmful especially during cold water immersion. The observed decrease of chloride levels is connected with the decline of sodium. We also found a non-significant decrease of potassium levels as reported by ROUGIER and BABIN (1975) who found a slight hypokaliemia after the 1500 m crawl – swimming, or (DOBREV *et al.* 1969) described very low serum potassium levels in athletes having swum 30,000 m.

### Renal function tests

Winter swimming was connected with a downward tendency in urea levels which is consistent with previous findings. SIEMS *et al.* (1999) described a decrease in uric acid to nearly half of the initial value after a single, controlled winter swimming session. The aforementioned effect was not explained just by increased uric acid excretion into urine and SIEMS *et al.* (1999) hypothesized that this may result from oxidative stress connected with hypothermic conditions. However, one day after exposure, uric acid levels returned to normal, supporting the hypothesis of the single, short term negative cold water immersion mechanism. We

did not observe a substantial decrease in uric acid levels, nor other renal parameters. This can be explained by activation of anti-oxidative stress mechanisms such as higher initial reduced glutathione (GSH) or superoxide dismutase (SOD) as described by SIEMS *et al.* (1999) against repetitive moderate cold induced stress during winter swimming.

### Liver function tests

We found no significant changes in plasma enzymes such as AST, ALT, GGT and LDH after regular winter swimming. Short-term cold water exposure (SIEMS *et al.* 1999) resulted in elevation of oxidative stress together with accelerated free radicals lipid peroxidation. It is well documented that oxidative stress along with lipid peroxidation may lead to mitochondrial dysfunction and result in hepatotoxic activity (YUAN & KAPLOWITZ 2009; HAN *et al.* 2006). The non-significant changes of liver enzymes subsequent to regular winter swimmers, as presented in this study, may indicate that short - term oxidative stress connected with cold water immersion described by (SIEMS *et al.* 1999) has induced a long-term adaptive response and improved antioxidative hepato-protection. This is only speculative because we have not measured antioxidative parameters.

### Serum proteins, cortisol

Proteins are an important component of plasma maintaining inner vascular pressure. They are divided into numerous fractions: albumin, globulin, fibrinogen, glycoproteins, lipoproteins and others. Plasma proteins undergo constant renewal and degradation. The protein concentration in the blood depends on its supply from food, synthesis in the liver and cells of the reticuloendothelial system, and the degree of protein loss by the kidney, gastrointestinal tract, skin, and lungs. The total protein level in the blood is the sum of albumin and globulins. Measuring the concentration of protein in the plasma of winter swimmers may indicate the level of resistance. In our study, an increased rate of albumin and A/G indicator is associated with the building up of winter swimmers' resistance against cold water after winter swimming. Immunoglobulins are a heterogeneous group of proteins of the immune system. Immunoglobulins or immune antibodies are produced by one of the white blood cell populations, namely, B lymphocytes. According to STOCKS *et al.* (2004), winter swimming does not have any effects on plasma osmolality, the total level of protein in the blood serum or electrolytes, which suggests that immersion in cold water is associated with a shift of extracellular fluid to the interstitial area. However, TELEGLÓW

*et al.* (2015) previously showed that after a break in winter swimming, there was a decrease in the concentration of total protein, albumin and beta-1 globulin. We found a considerable decrease in gamma globulin plasma concentration accompanied by a fall in IgG. As reported by (GLEESON *et al.* 2000) who investigated changes in salivary IgA and IgM during 1 year among Antarctic winter expeditioners, salivary IgA and IgM decreased in the first 4 months of observation and after that, increased to maximal values. These results were not connected with the amplified incidence of infections and did not support the hypothesis of cold-induced immunosuppression. (CASTELLANI *et al.* 2002a) supported the thesis that moderate cold exposure has no effect on the innate component of the immune system. Cold exposure causes the activation of the sympathetic nervous system described by increased plasma norepinephrine concentrations and alpha-adrenergic peripheral vasoconstriction.

JANSKY *et al.* (1996) showed that repeated cold water immersions did not influence IgG, IgA and or IgM production in humans. On the other hand, the cold pressor test as an acute cold stress increases both salivary volume and sIgA concentration accompanying cardiovascular responses mediated predominantly by  $\alpha$  - adrenergic receptors (WILLEMSSEN *et al.* 1998). It is widely recognized that IgA, because of its dominance in the immune system, is the first line of defense against harmful environmental factors. In our study, we found a slight increase in IgA after winter swimming and HSV IgM (cause unknown), a slight decrease in gamma globulin including IgG,  $\alpha$  - 2 globulin which is associated with acquired resistance in winter swimmers. Epidemiological data presented by BRENKE (1990) indicate a 40% decrease in respiratory tract infections due to regular cold water swimming.

Serum cortisol, a stress induced hormone, can modulate immune response by suppressing and controlling inflammation with glucocorticoid receptor expression on lymphocyte cells. The data concerning cold-related changes in cortisol secretion are equivocal (WILKERSON *et al.* 1974; FRANK *et al.* 1997; MARINO *et al.* 1998; GAGNON *et al.* 2014). This is probably due to a non-objective testing methodology. The level of serum cortisol can be affected by numerous factors such as time of day, sleep deprivation, etc. (CASTELLANI *et al.* 2002b). We found no differences in plasma cortisol concentrations in our study.

Despite the wide use of low temperatures in many aspects of life, the data concerning long-term cold water swimming are subject to limited research. The present findings indicate several positive adaptive mechanisms to regular cold water immersion. A further survey concerning anti-

oxidant capacity and hormone regulation following low-temperature exposition is needed.

## Acknowledgments

The authors would like to thank the winter swimmers from the Krakow Walrus Club “Kaloryfer” – “The Heater”.

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